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Key Advances in Medicine

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ATRIAL FIBRILLATION IN 2012

Advances in catheter-ablation treatment of AF

Andrea Natale

Atrial fibrillation (AF) is increasingly being treated using percutaneous or surgical procedures. During 2012, various key studies improved our understanding of which forms of AF respond best to catheter ablation, how to optimize the ablation procedure and postprocedural care, and which patients should receive medical therapy.

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Several important studies relating to the treatment of atrial fibrillation (AF) with percutaneous or surgical procedures were published in 2012.[1–5] In the FAST randomized trial[1] conducted in two European centres, minimally invasive surgical ablation was compared with radiofrequency catheter ablation (RFCA; Figure 1) in 124 patients with drug-refractory AF and either previous failed catheter ablation (67%) or left atrial dilatation plus additional risk factors, such as hypertension (33%). The primary end point was freedom from recurrent atrial arrhythmia lasting >30 s in the absence of antiarrhythmic drugs, and was reached in 36.5% and 65.6% of patients allocated to RFCA or surgical ablation, respectively (P=0.0022).[1] Notably, major adverse events were more common with surgical ablation than with RFCA (34.4% versus 15.9%; P=0.027).[1] The investigators concluded that surgical ablation was superior to RFCA for the maintenance of sinus rhythm at 1 year in this group of patients, but that surgical ablation was associated with a higher risk of procedural adverse events.

The results of the FAST study,[1] albeit clinically relevant, should be interpreted with caution. The RFCA technique was heterogeneous across the enrolling centres, with the use of a 4 mm solid-tip catheter in one centre and a 4 mm irrigated-tip catheter in another, coupled with the discretionary use of left atrial substrate modification on top of pulmonary vein isolation.[1] Additionally, when translating the rate of success and complications into actual treatment effects, the benefit of surgical ablation is highly attenuated. For instance, approximately 294 more patients in every 1,000 treated with surgical ablation will maintain sinus rhythm at 1 year, at a cost of 200 more patients experiencing serious periprocedural complications, including pneumothorax, thromboembolism, and major bleeding.[1] In summary, the FAST trial[1] suggests that surgical ablation might improve the 1-year freedom from recurrent arrhythmia compared with RFCA in patients with an enlarged left atrium and hypertension or in those who have already failed a RFCA procedure, but the risk–benefit ratio of surgical ablation seems to be unfavourable.

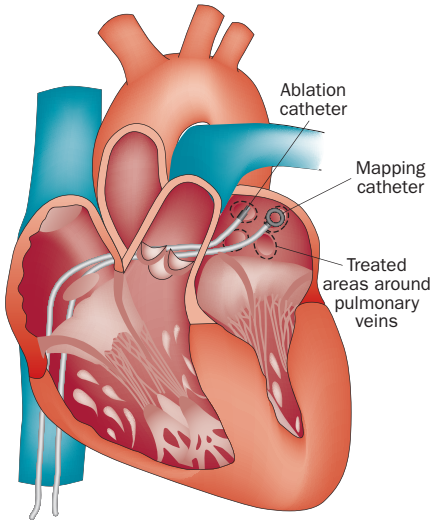

Figure 1 | Pulmonary vein isolation by catheter ablation to treat atrial fibrillation.

Data from the MANTRA-PAF randomized trial[2] are also likely to shape new recommendations for RFCA of AF. In this study, 294 patients with paroxysmal AF and no previous antiarrhythmic-drug exposure were randomly allocated to RFCA or antiarrhythmic drugs (class Ic or III). The primary end point was AF burden, measured as both cumulative and per-follow-up-visit burden (3, 6, 12, 18, and 24 months with 7-day Holter monitoring). AF burden was not significantly different between patients treated with RFCA and those receiving antiarrhythmic drugs up to 18 months, but was significantly lower in the RFCA arm at 24 months (90th percentile 9% versus 18%, respectively; P=0.007).[2]

The results of the MANTRA-PAF trial,[2] although positive, are not impressive. The adoption of inconsistent and obsolete ablation techniques, such as circumferential ablation without confirmation of pulmonary vein isolation with a circular mapping catheter, might explain the suboptimal results achieved with RFCA in this trial. RFCA was shown to have a similar rate of complications as antiarrhythmic-drug therapy (17% versus 15%). A remarkably high number of adverse events—two strokes or transient ischaemic attacks, three cardiac tamponades, and one perforation during the trans-septal puncture—were reported in the RFCA group. Again, the complications reported in the RFCA arm might plausibly have been minimized if state-of-the-art ablation strategies were consistently adopted in the trial, including performing procedures without therapeutic warfarin discontinuation,[6] and using intracardiac echocardiography to assist the trans-septal puncture, catheter manipulation, and radiofrequency power titration. Also, 36% of patients initially assigned to antiarrhythmic-drug therapy crossed over to RFCA. Therefore, the actual benefit of RFCA reported in the MANTRA-PAF trial[2] was likely to be an underestimate because outcomes were evaluated according to the intention-to-treat principle.

The role of catheter ablation in patients with more-challenging forms of AF, such as those with either persistent or long-standing persistent AF, has also been actively investigated in studies published in 2012. Tilz and colleagues reported their long-term results with RFCA of long-standing persistent AF.[3] The ablation technique adopted in this

Key advances

- Surgical ablation might be considered in patients with atrial fibrillation (AF) who have already failed a catheter-ablation procedure, or have left atrial dilatation and hypertension[1]
- Pulmonary vein isolation is superior to antiarrhythmic-drug therapy for the first-line treatment of paroxysmal AF[2]
- Pulmonary vein isolation alone is largely ineffective for the maintenance of long-term sinus rhythm in patients with nonparoxysmal AF[3]
- Dabigatran should not be recommended for periprocedural anticoagulation in patients undergoing catheter ablation of AF[4]
- A novel, computational-mapping technique to identify and target localized sources of AF has shown promising results in a small, nonrandomized study[5]

study included pulmonary vein isolation and, if the patients did not resume normal sinus rhythm, external cardioversion. After a 30 min waiting period, if no further spontaneous AF was seen, the procedure was terminated, whereas if AF recurred, the focal trigger was ablated. If cardioversion was unsuccessful, areas with complex fractionated electograms were targeted, and linear lesions were delivered to terminate macro-re-entrant atrial tachycardias. After follow-up (median 56 months), sinus rhythm was maintained in 20.3% of patients after a single procedure, and reached 45.0% after multiple procedures (overall median two procedures, range one to five procedures). The main message that arises from this study is that pulmonary vein isolation alone is largely insufficient to achieve satisfactory long-term, arrhythmia-free survival in patients with long-standing persistent AF,[3,7] as shown in multiple previous studies.[8] However, the amount of additional ablation necessary in these patients to increase the procedural success is still a matter of controversy. To date, the approach in most institutions is to provide extensive ablation targeting the left atrial substrate and areas demonstrating nonpulmonary vein triggers,[9] either spontaneously or after induction with pacing or drug challenges (such as with isoproterenol).

Lakkireddy and colleagues evaluated the safety of dabigatran for periprocedural anticoagulation in patients undergoing AF ablation in a multicentre, observational study.[4] A total of 290 patients (145 taking dabigatran and 145 matched patients taking uninterrupted warfarin) were included in the analysis. Three thromboembolic complications (2.1%) occurred in the dabigatran group, whereas no thromboembolic events occurred in the warfarin group. Furthermore, the dabigatran group had a significantly higher rate of major bleeding (6% versus 1%), total bleeding (14% versus 6%), and composite of bleeding and thromboembolic complications (16% versus 6%; $P = 0.009$) than the warfarin group. Periprocedural use of dabigatran was an independent predictor of bleeding or thromboembolic complications (OR 2.76, $P = 0.01$).[4] The results of this study clearly discourage the use of dabigatran for periprocedural anticoagulation in patients undergoing AF ablation.

A novel and promising computational-mapping technique has been developed, which can identify localized sources of AF that are supposed to correspond to organised re-entrant circuits ('rotors') or focal impulses seen in animal studies of AF pathophysiology.[10] In a multicentre study, Narayan and colleagues evaluated the benefit of ablation of these localized sources (termed 'focal impulses and rotor modulation'; FIRM) in a consecutive series of 92 patients, predominantly with persistent AF (72%), who underwent a total of 101 ablation procedures.[5] Patients were allocated in a 1:2 ratio to either ablation of localized rotors or focal impulses ($n = 36$) or conventional ablation ($n = 71$). Notably, the trial was not randomized and the criteria underlying the allocation to either FIRM or conventional ablation were not fully disclosed. Localized rotors or focal impulses were found in 97% of patients in the FIRM-guided group, and ablation of these sites resulted in AF termination or consistent slowing in 86% of these individuals. After follow-up (median 273 days after a single procedure), FIRM-guided patients achieved higher freedom from recurrent AF than those treated with conventional ablation (82.4% versus 44.9%; $P < 0.001$), as assessed using implantable loop recorders or interrogation of pacemaker–defibrillators with AF-detection algorithms. Overall, these data are very promising. The extent to which these results are reproducible by other institutions and, most importantly, are applicable to more-challenging forms of AF (such as long-standing persistent AF) warrants further investigation.

In conclusion, we have learned several important lessons from studies published in 2012 in the field of AF ablation. Pulmonary vein isolation has been definitively shown to be superior to antiarrhythmic drugs as a first-line therapy in patients with paroxysmal AF. In patients with nonparoxysmal AF, especially those with long-standing persistent AF, pulmonary vein isolation alone is largely ineffective in achieving long-term freedom from recurrent arrhythmia. The importance of targeting areas beyond the pulmonary veins in these patients has been consistently reported, and cannot be overemphasized. The optimal strategy to achieve long-term freedom from recurrent arrhythmia in these patients is still a matter of controversy. Further studies are warranted to test whether additional nonpulmonary vein areas should be targeted with extensive catheter ablation, surgical procedures, or with FIRM-guided ablation.

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CORONARY ARTERY DISEASE IN 2012

Revising common beliefs in the management of stable CAD

Roberto Ferrari

New findings published in 2012 have challenged common beliefs about stable coronary artery disease. Studies have shown that β-blockers and n-3-polyunsaturated fatty acids have no impact on prognosis, that percutaneous coronary intervention is not always the best option, and that women do not have worse cardiovascular outcomes than men.

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Medicine is evolving rapidly, and cardiology is no exception. Over the past 3 decades, lifespan has increased by 8 years, to which cardiology has contributed at least 6 years.[1] Much of this success has resulted from the development of new therapeutic strategies. However, in addition to testing new ideas, it is important that clinical investigators also assess the validity of older ideas in the contemporary setting. Indeed, four key studies published in 2012 have provided results that challenge accepted concepts regarding the management of patients with stable coronary artery disease (CAD).

In October 2012, a follow-up report of the REACH registry showed that β-blockers are not associated with lower rates of cardiovascular events in patients with CAD with or without myocardial infarction (MI).[2] Of the 44,708 REACH registry patients who had either known prior MI, CAD without MI, or risk factors for CAD but no known CAD at baseline, 21,860 were included in this analysis. In all three propensity-score-matched cohorts, β-blocker use was not associated with reduced risk of either the primary outcome (cardiovascular death, nonfatal MI, or nonfatal stroke) or the secondary outcome (primary outcome plus hospitalization for atherothrombotic events or revascularization) compared with no β-blocker use.

These findings are important for several reasons. Firstly, in the latest versions of the US and European guidelines, the recommendations for long-term use of β-blockers in patients with CAD were downgraded, except for patients who also had heart failure or acute coronary syndromes. Despite this change in recommendations, and on the basis of evidence extrapolated from post-MI studies dating from the era before contemporary reperfusion and medical therapy (median publication date 1982), cardiologists continue to consider β-blockade for patients with CAD. Secondly, the CAD phenotype has changed over the years. A reperfused, viable myocardium is less arrhythmogenic, and ejection fraction is less affected by the timing of revascularization, than necrotic, scarred heart muscle. Drugs commonly used today—aspirin, statins, and angiotensin-converting enzyme (ACE) inhibitors—exert beneficial effects on the coronary artery endothelium with reduction in atherosclerosis progression and plaque stabilization. Therefore, the additional prognostic role of β-blockers is limited to reduction of heart rate and blood pressure. Findings from the REACH registry show that this role is not enough to improve prognosis in daily clinical practice, possibly because of suboptimal dosage owing to adverse effects. Finally, the fact that these data are generated by a registry is important. Clearly, in the absence of clinical trials, well-designed, contemporary, observational studies are useful to reconfirm or challenge the value of previously validated treatments.

New results from the ORIGIN trial[3] on the use of n-3-polyunsaturated fatty acids (PUFA) in patients with dysglycaemia have challenged another common belief—the prognostic benefit of PUFA in cardiovascular disease. In previous studies, reported in 1999 and 2008, PUFA supplementation was found to be associated with a modest reduction in fatal and nonfatal cardiovascular events, both after MI[4] and in the setting of heart failure,[5] in patients with similar glycaemic conditions to those in the ORIGIN study. By contrast, in 2012, the ORIGIN investigators reported that 1 g PUFA daily did not confer additional protection against the primary end point of cardiovascular death, compared with placebo (olive oil), in patients with diabetes mellitus or prediabetes at high risk for cardiovascular disease.[3] The ORIGIN trial was a large study with a high number of events, a long duration of follow-up, and excellent adherence to study medication, and so the results are reliable.

The discrepancy between the findings from the ORIGIN trial and the previous studies described above could have resulted from differences in disease characteristics (the ORIGIN trial involved fewer patients with arrhythmias, who could benefit from the potential antiarrhythmic effects of PUFA), fatty acid content of adipose tissue in patients, or possibly from a beneficial effect of the ORIGIN trial placebo (olive oil). Moreover, the high rate of concomitant cardioprotective therapies in the ORIGIN study might have reduced the incidence of death from cardiovascular causes and, therefore, the statistical power to detect any effect of PUFA. This hypothesis is supported by the 2010 trial report of a study of

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Key advances

- β-Blockers are probably not useful in patients with, or those at risk of, stable coronary artery disease (CAD)[2]
- n-3-Polyunsaturated fatty acid supplements are probably not useful in patients with, or those at risk of, stable CAD[3]
- The functional significance of stenoses should be assessed before percutaneous coronary intervention is performed in patients with stable CAD[8]
- Women with stable CAD have the same cardiovascular risk as men[10]

PUFA supplementation in patients with MI who were receiving contemporary cardioprotective therapies—the Alpha Omega Trial Group did not find any beneficial effect of PUFA supplementation in these patients.[6] If true, and considering that the effects shown in the previous trials were marginal, should PUFA supplementation continue to be prescribed on top of the recommended preventative therapies to further improve CAD prognosis? Would a meta-analysis of all existing data help us to answer this question? Probably not, given that the ancillary problem of the background therapy would remain. However, three large clinical trials are still ongoing and will, in all likelihood, produce the final word.[3]

Since the advent of percutaneous coronary intervention (PCI), visual assessment of the severity of coronary stenosis has been used to guide revascularization in CAD. In 2007, the COURAGE trial investigators reported that PCI, guided by visual assessment, was not of additional benefit in the initial management of low-risk patients with stable CAD who were receiving optimal medical therapy.[7] In 2012, however, FAME-2 demonstrated the superiority of a strategy involving PCI guided by pressure-derived fractional flow reserve (FFR ≤0.8) plus best available medical therapy over best available therapy alone in such patients.[8]

The plan for FAME-2 was to enrol 1,632 patients with a 2-year follow-up, but the trial was terminated after a mean of 7 months because of a reduced need for urgent revascularization (a component of the primary end point) in the intervention group. FAME-2 was criticized for selection bias with regard to revascularization, lack of objective confirmation of ischaemia, cost-effectiveness of the FRR approach, too short follow-up, and too few hard events overall. Nevertheless, the importance of urgent revascularization and unplanned hospitalization, and the associated costs, should not be underestimated. The results of FAME-2 provide a new scenario for stable CAD—the potential to identify lesions that need to be treated on the basis of a robust pathophysiological argument, that is, the likelihood that the stenosis is functionally significant and causes ischaemia. These findings should have great impact, given that less than half of patients undergo noninvasive stress testing before elective PCI.[9] In my opinion, however, the most-important message from the results of FAME-2 and the COURAGE trial is a more philosophical one that should guide all medical attitudes—act only when necessary and not just for the sake of it.

Finally, a 2012 report from the ongoing international registry CLARIFY challenged ideas surrounding the role of gender in CAD.[10] In total, 33,285 outpatients with stable CAD who were receiving standard management were enrolled in CLARIFY; 22.6% of these individuals were women.[10] Substantial differences in presentation and management of men and women were apparent. Women were older and had a higher risk-factor burden; they had less access to noninvasive and invasive investigations, leading to a lower rate of revascularization; and they did not receive statins or β-blockers as often as men. Surprisingly, despite the aforementioned differences, the rates of cardiovascular outcomes at 1 year were similar in men and women, even after adjustments for potential confounders. Moreover, premenopausal women, or those at low risk and without a history of MI and revascularization, had a better cardiovascular prognosis than men with a similar profile.

These data are certainly reassuring for women and challenge the common—but unproven—belief that postmenopausal women lose the protective effect of oestrogen and have poorer cardiovascular outcomes than men. Despite the good news, however, the data from CLARIFY warrant further consideration. The CLARIFY registry involved a large cohort from a broad geographical area (45 countries, excluding the USA) and yet only 22.6% of enrolled patients were women. This statistic contradicts epidemiological data showing that the prevalence of CAD is the same in both sexes, if not higher in women with angina.[10] Therefore, in CLARIFY, either fewer women were seen by the physicians, or the physicians themselves made a selection bias (even though they were encouraged to enrol consecutive patients). Both potential scenarios are in line with the reported differences in cardiovascular care between men and women in CLARIFY—underdiagnosis and less treatment in women—and reinforce the need to modify physician and patient behaviour and increase awareness of the prevalence of CAD in women. Physicians should pay more attention to possible cardiovascular symptoms in women, which are frequently atypical, and should not perceive women to be at a lower risk than men. Women should probably be more concerned about their cardiovascular conditions and visit physicians more often.

In 2012, data from trials and registries have challenged previous concepts and beliefs related to the management of stable CAD. Such continuous efforts to conduct research and question commonly held beliefs is what makes the medical profession worthwhile.

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HEART FAILURE IN 2012

Trial data resolve gaps in evidence-based treatment

Adriaan A. Voors

In the 2012 ESC guidelines for the management of heart failure, various gaps in the clinical evidence base were identified. Four studies published in 2012 go some way to resolving this data deficit, and treatment recommendations can now be updated accordingly.

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In the spring of 2012, the ESC updated their 2008 guidelines for the diagnosis and treatment of acute and chronic heart failure (HF).[1] The most-important changes from the previous version related to expanded indications for the use of the mineralocorticoid-receptor antagonist eplerenone, the sinus-node inhibitor ivabradine, and for cardiac resynchronization therapy. A specific section of these guidelines was dedicated to gaps in evidence. Shortly after publication of the guidelines, four studies partly resolved some of these important gaps.

One of the identified areas of uncertainty was the use of anticoagulants in patients with HF who were in sinus rhythm. Because HF is associated with a hypercoagulable state, formation of left ventricular thrombus, and cerebral embolism, anticoagulants might be beneficial in these patients. The 2012 ESC guidelines state that "Other than in patients with atrial fibrillation, there is no evidence that an oral anticoagulant reduces mortality–morbidity compared with placebo or aspirin."[1] The WARCEF trial[2] ended this uncertainty. Investigators in WARCEF randomly allocated 2,305 patients with HF who were in sinus rhythm to aspirin (325 mg daily) or warfarin (target international normalized ratio 2.0–3.5). No significant changes were observed in the time to the first event in a composite end point of ischaemic stroke, intracerebral haemorrhage, or death from any cause (mean follow-up 3.5 years).[2] The benefit of warfarin in reducing the rate of ischaemic stroke was offset by an increase in the rate of major bleeding. One criticism of the design of this study is the dose of and indication for aspirin. Only 48% of the patients had a previous myocardial infarction, and no strong indication for the use of aspirin existed for the remaining patients. A lower dose of aspirin than was used in the trial (or no aspirin in patients without a clear indication) might have reduced the risk of bleeding compared with warfarin even further. Finally, subgroup analyses to identify potential groups of patients that might still benefit from warfarin are, so far, not available. After the WARCEF trial,[2] the wording of the ESC guidelines could now be changed to state that "There is evidence that an oral anticoagulant does not reduce morbidity or mortality compared with aspirin in patients with HF who are in sinus rhythm."

A second area of uncertainty presented in the 2012 ESC guidelines was the efficacy and safety of ultrafiltration in patients with acute decompensated HF. The guidelines currently state that "Venovenous isolated ultrafiltration is sometimes used to remove fluid in patients with HF, although is usually reserved for those unresponsive or resistant to diuretics."[1] The CARRESS-HF[3] investigators studied the safety and efficacy of ultrafiltration compared with pharmacological therapy in 188 patients with acute decompensated HF complicated by worsening renal function and persistent congestion. Contrary to general expectations, ultrafiltration did not increase weight loss, and was associated with a significantly higher increase in the serum creatinine level than drug therapy. Additionally, a higher percentage of patients in the ultrafiltration group than in the pharmacological-therapy group had a serious adverse event (72% versus 57%; $P = 0.03$).[3] After CARRESS-HF,[3] the ESC guidelines could be reworded to state that "Venovenous isolated ultrafiltration should not be used to remove fluid in patients with HF and signs of congestion and worsening renal function." However, the effects of ultrafiltration in patients with acute, decompensated HF who are resistant to diuretics remain to be established.

A third important gap is in the evidence-based treatment of patients who have HF with a preserved left ventricular ejection fraction (HFpEF or diastolic HF). Similarly to the 2008 ESC guidelines, the 2012 version states that "No treatment has yet been shown, convincingly, to reduce morbidity and mortality in patients with HF-PEF."[1] Large, randomized clinical trials with angiotensin II-receptor blockers and angiotensin-converting-enzyme inhibitors have failed to demonstrate a reduction in mortality and morbidity.[4] A combined angiotensin II-receptor and neprilysin inhibitor (LCZ696) has now been shown to reduce blood pressure.[5] Neprilysin degrades biologically active natriuretic peptides, thereby improving myocardial relaxation, reducing hypertrophy, and stimulating diuresis, natriuresis, and vasodilatation. In PARAMOUNT,[6] 234 patients with HFpEF were randomly allocated to LCZ696 or valsartan. LCZ696 reduced the level of the *N*-terminal prohormone of B-type natriuretic peptide to a greater extent than did valsartan at 12 weeks, and was associated with left atrial reverse remodelling and improvement in NYHA classification at 36 weeks.[6] Whether these promising findings will translate into a reduction in morbidity and mortality will have to be tested in a much larger, phase III, randomized clinical trial. Consequently, the recommendations in the ESC guidelines cannot, as yet, be updated. Results of the TOPCAT trial[7] are expected in 2013. The TOPCAT study is a phase III randomized clinical trial on the effects of spironolactone versus placebo in 3,445 patients with HFpEF, which might become the first study to show a convincing reduction in morbidity and mortality in these patients.

Key advances

- In patients with heart failure in sinus rhythm, warfarin is not better than aspirin in reducing the combined end point of ischaemic stroke, intracerebral haemorrhage, or death from any cause[2]
- Venovenous ultrafiltration does not increase urinary output, but impairs renal function and increases adverse events in patients with acute heart failure, worsening renal function, and persistent signs of congestion[3]
- LCZ696, a novel dual angiotensin II-receptor and neprilysin inhibitor, improves symptoms and decreases left atrial volume in patients who have heart failure with preserved ejection fraction[6]
- Serelaxin, a human recombinant form of the naturally occurring hormone relaxin, improves dyspnoea and reduces cardiovascular mortality in patients with acute heart failure[9]

Finally, an important gap was identified in the evidence-based treatment of patients with acute, decompensated HF. The ESC guidelines state that "The treatment of acute HF remains largely opinion-based with little good evidence to guide therapy."[1] To date, no drug has been shown to improve both morbidity and mortality in acute, decompensated HF. In 2009, relaxin showed promising effects in a phase II, dose-finding study in patients with acute, decompensated HF.[8] Relaxin is a naturally occurring hormone, with vasodilatory properties through inhibition of the vasoconstrictive effects of endothelin-1 and stimulation of nitric oxide synthase. Additionally, relaxin has anti-inflammatory and antifibrotic effects, and improves renal blood flow. Therefore, the mode of action of serelaxin (recombinant human relaxin 2) is substantially different from that of nitrates. In the RELAX-AHF trial,[9] 1,160 patients with acute, decompensated HF, a systolic blood pressure >125 mmHg, and mild renal dysfunction were randomly allocated to serelaxin (30 μg/kg daily) or placebo. Serelaxin improved dyspnoea, but did not reduce the secondary end points of cardiovascular death, readmission to hospital for HF, or days alive out of hospital up to day 60.[9] However, serelaxin had multiple additional beneficial effects, such as a reduction in worsening HF, length of hospital stay, and end-organ damage. Also, despite increased use of intravenous diuretic drugs and vasoactive drugs, such as nitrates, significantly greater reductions in the signs and symptoms of congestion occurred in serelaxin-treated patients by day 2. Most importantly, serelaxin significantly reduced all-cause and cardiovascular mortality.[9] Therefore, serelaxin is the first drug that has conferred both morbidity and mortality benefits in these patients. However, neither all-cause nor cardiovascular mortality was a primary or secondary end point of the trial, but both were prespecified end points. Serelaxin might be a breakthrough in the treatment of acute, decompensated HF and, after the RELAX-AHF trial,[9] the ESC guidelines could include the statement that "Relaxin is the first drug that seems to improve both morbidity and mortality in patients with acute, decompensated HF."

In summary, 2012 was an important year in HF, when important gaps in evidence were resolved, and interesting novel therapies emerged for the treatment of patients with either HFpEF or acute, decompensated HF. In both areas, evidence-based therapies are desperately needed.

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Competing interests
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INTERVENTIONAL CARDIOLOGY IN 2012

We are 'shocked', 'frozen', and 'freed' by new data

Roxana Mehran

In 2012, results from three studies in interventional cardiology have enhanced our knowledge on the best practices to improve clinical outcomes. These trials were focused on treatment safety as well as efficacy. The optimal therapeutic strategy for patients undergoing percutaneous coronary intervention continues to evolve.

Mehran, R. *Nat. Rev. Cardiol.* **10**, 68–70 (2013); published online 15 January 2013; doi:10.1038/nrcardio.2012.200

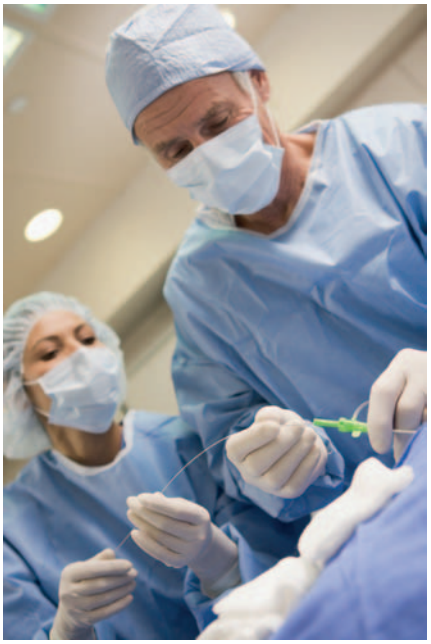
Evidence-based guidelines are the cornerstone of clinical practice. Three clinical studies published in 2012, IABP-SHOCK II,[1] ARCTIC,[2] and FREEDOM,[3] have provided us with a wealth of knowledge and data, substantially contributing to our goal to improve the clinical practice of interventional cardiology. These studies have employed ancillary devices for haemodynamic support and platelet function

testing to improve treatment, the identification of patients at risk of adverse events, and the evaluation of outcomes.

In the multicentre IABP-SHOCK II trial,[1] patients with acute myocardial infarction complicated by shock were randomly allocated to either percutaneous coronary intervention (PCI) with intra-aortic balloon pump (IABP) insertion and medical treatment ($n = 119$), or PCI and medical treatment only ($n = 123$). The findings of this study have important implications for clinical cardiology. IABP is regarded as the preferred treatment for these patients but, in general, this view is supported only by registry data. Therefore, the opportunity to review findings from a randomized trial on this treatment was highly anticipated. Surprisingly, the investigators found that the addition of IABP insertion to the treatment regimen did not significantly reduce 30-day mortality, the primary end point (39.7%), compared with the control group (41.3%). Notably, the IABP was inserted after the PCI procedure in most patients; therefore, whether IABP use before PCI is beneficial remains unknown. The value of haemodynamic support in patients with cardiogenic shock should not be underestimated on the basis of these data.

The subsequent ARCTIC trial[2] focused on the optimization of antiplatelet therapies after PCI. The investigators of the ARCTIC trial hypothesized that the rate of severe cardiovascular complications, the primary end point of the study, at 1-year follow-up might be reduced with aspirin and clopidogrel dual antiplatelet therapy (DAPT) if the drug doses were adjusted by biological monitoring.[2,4] Conversely, they also proposed that the complication rate would increase if DAPT were interrupted by discontinuation of clopidogrel after 1 year, and continuation of aspirin alone for an additional 6 months, compared with maintaining DAPT during this time. However, no significant difference was found in the clinical outcomes of patients receiving platelet-function monitoring compared with those receiving standard, unmonitored antiplatelet therapy.

This randomized trial had a well-defined control group (receiving 75 mg clopidogrel with aspirin daily), but the monitored-treatment arm had a suboptimal design and was not well-defined. The highly heterogeneous pool of participants in the monitored-treatment group reduced the power of this study. Furthermore, 150 mg clopidogrel daily was used in the majority of



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patients, which was a substandard choice of dose for two reasons: 150 mg clopidogrel had previously been shown to be ineffective,[5] and not to produce a significant increase in platelet inhibition compared with a 75 mg daily dose.[6] Importantly, the event rate was low in the control group, which might reflect a low-risk profile for the entire study population and could, therefore, be responsible for the lack of a difference in risk reduction between the study arms. The fact that ischaemic outcomes were not improved by monitoring and adjusting the dose of antiplatelet therapy is consistent with finding from previous randomized trials, but contradicts registry data indicating that ischaemic risk and residual platelet activation are increased in patients receiving oral antiplatelet therapy, such as oral P2Y12 inhibitors.[7] The ARCTIC data show that bleeding events were reduced by monitoring platelet inhibition, indicating that DAPT is safe and that platelet-function testing can be used to avoid bleeding. Future studies need to focus on platelet inhibition to attenuate bleeding events rather than to reduce the rate of ischaemic events.

Investigators in the WOEST study,[8] the findings of which were presented at the 2012 ESC Congress but have not yet been published in a peer-reviewed journal, also assessed antithrombotic agents in the setting of PCI. This study was the first randomized trial in which two antiplatelet regimens—clopidogrel with or without aspirin—were assessed in patients taking oral anticoagulant therapy (OAC) or in those with chronic indications for OAC who were undergoing PCI. No previous research had addressed whether all three agents—aspirin, clopidogrel, and warfarin—should be coadministered in these patients. One limitation of triple therapy is that the incidence of bleeding complications could increase, thereby necessitating discontinuation of aspirin, clopidogrel, or both, and that the therapy could trigger a subsequent coronary event. On the basis of their results, the investigators concluded that omitting aspirin treatment in patients receiving warfarin who will undergo stenting might reduce the risk of bleeding by half, and reduce mortality, without an effect on the rate of stent thrombosis. However, this study lacked a sufficient number of participants; therefore, the results are not definitive and a larger trial must be conducted to test these findings. Until such a study is properly executed and completed, the combination of clopidogrel plus OAC should be accepted as the most-reasonable treatment option available for patients with a chronic indication for OAC and those who have undergone coronary stent implantation.

The FREEDOM trial[3] was undertaken to compare the current revascularization strategies for patients with diabetes mellitus. In previous studies, better outcomes were reported with CABG surgery than with PCI, but these procedures were performed using bare-metal stents and balloon angioplasty. In the FREEDOM trial, conducted from 2005 to 2010 at 140 international centres, 1,900 patients with diabetes (mean age 63.1 ± 9.1 years, 29% women, and 83% with three-vessel coronary artery disease [CAD]) were assigned to revascularization treatment with either PCI using drug-eluting stents or CABG surgery. The patients were followed up for a minimum of 2 years (median duration among survivors was 3.8 years). The primary end point was a composite of death from any cause, nonfatal myocardial infarction, or nonfatal stroke. All patients were prescribed the currently recommended medical therapies for the control of LDL cholesterol, systolic blood pressure, and glycated haemoglobin (HbA_{1c}).

The primary end point occurred significantly more frequently in the PCI-treated group than in those who underwent CABG surgery, with a 5-year event rate of 26.6% and 18.7%, respectively ($P = 0.005$). These results were driven by significant differences in the rate of both myocardial infarction (13.9% versus 6.0%; $P < 0.001$) and death from any cause (16.3% versus 10.9%; $P = 0.049$). The 5-year rate of stroke was higher in the CABG-surgery group than

Key advances

- In the IABP-SHOCK II trial,[1] intra-aortic balloon pump (IABP) insertion after percutaneous coronary intervention (PCI) did not reduce 30-day mortality, but whether IABP insertion before PCI is beneficial remains unknown
- In the ARCTIC trial,[2] monitoring of platelet reactivity during dual or triple antiplatelet therapy did not confer a marked improvement in outcomes compared with treatment without monitoring
- In the FREEDOM trial,[3] revascularization with CABG surgery was superior to PCI in patients with diabetes mellitus for all outcomes except stroke

in the PCI-treated group (5.3% versus 2.4%; $P = 0.03$). The event curves for stroke diverged 2–3 years after randomization. In patients with diabetes and advanced CAD, CABG surgery was superior to PCI in reducing the rate of late myocardial infarction and death. CABG surgery was associated with a significantly lower major adverse event rate, but a higher overall rate of stroke and other perioperative morbidity, than PCI.

The FREEDOM trial[3] has provided us with definitive data that CABG surgery is the best choice for revascularization therapy in patients with diabetes and three-vessel CAD in the current PCI era. This landmark study is the first trial to show directly that a protective effect could be conferred by bypassing the entire proximal segment of diseased coronary arteries, rather than selective treatment of the most-obstructed spots with drug-eluting stents. The diffuse characteristics of CAD in patients with diabetes, and the prothrombotic properties of blood cells and platelets in these patients, underscore the clinical importance of these findings. Further research is essential to characterize the effects of second (and subsequent) generations of drug-eluting stents in the successful treatment of patients with diabetes. In addition, in the FREEDOM trial, successful treatment of coronary risk factors, including LDL cholesterol, HbA_{1c}, and arterial blood pressure, was achieved in only few patients. Therefore, future research should also target successful risk-factor modification over a long time period.

Overall, these studies have provided great insight into the clinical practice of interventional cardiology. Further studies are required to optimize treatment strategies for patients, with or without diabetes, who have cardiogenic shock, or CAD and are undergoing PCI.

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LIPIDS IN 2012

HDL cholesterol studies—more of the same?

Jean-Pierre Després

Studies published in 2012 in the field of HDL research have provided further evidence suggesting that a low HDL-cholesterol level, in the absence of related lipid or nonlipid risk factors, is not associated with increased risk of coronary heart disease.

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Analyses of data from prospective observational cohorts published over the past 30 years have shown that increased levels of circulating HDL cholesterol are associated with a reduced risk of coronary heart disease events.[1] Furthermore, numerous preclinical studies have revealed that HDL particles have properties that could provide protection against the development of atherosclerosis.[2] On the basis of this evidence, raising the level of HDL cholesterol has generally been considered a legitimate strategy to reduce cardiovascular risk through reduced progression, or even regression, of atherosclerotic plaque. Considerable efforts have been devoted to the development of HDL-cholesterol raising therapies in the hope that they will add to the well-documented benefits of statin-mediated lowering of LDL cholesterol. In this article, four important papers published in 2012 that have reinforced our understanding about the role of HDL particles and HDL cholesterol in cardiovascular risk will be highlighted.

One pharmacological approach that produces considerable increases in the circulating level of HDL cholesterol is inhibition of cholesteryl ester transfer protein (CETP), an enzyme that promotes net mass transfer of cholesteryl esters from HDL particles to apolipoprotein B-containing lipoproteins.[2,3] Despite promising preclinical results, torcetrapib—the first CETP inhibitor tested in clinical trials—was not found to reduce the size of atherosclerotic plaques in imaging trials.[4] Furthermore, torcetrapib was reported to increase mortality and cardiovascular events in the large trial ILLUMINATE.[5] This phenomenon could be related, among other factors, to an increase in blood pressure and aldosterone

levels. Nevertheless, *post-hoc* analyses of results from torcetrapib trials combined with preclinical data suggested that the off-target effects of torcetrapib were probably unrelated to CETP inhibition.[3] Therefore, the hypothesis that CETP inhibition could reduce, or even reverse, atherosclerotic vascular disease remained to be tested.

Another CETP inhibitor, dalcetrapib, was assessed in the DAL-OUTCOME study and the results published in November 2012.[6] Importantly, this compound was found to have little effect on the level of LDL cholesterol while increasing HDL-cholesterol concentration.[3] These findings provided the appropriate experimental conditions to test the clinical benefit on cardiovascular outcomes of specifically raising the HDL-cholesterol level with dalcetrapib. In the DAL-OUTCOME study,[6] 15,871 patients who had experienced a recent (median 61 days) acute coronary syndrome were randomly assigned to receive dalcetrapib (600 mg per day) or placebo on top of the best evidence-based clinical management. During the trial, HDL-cholesterol levels increased from baseline by 4–11% in the placebo group and by 31–40% in the dalcetrapib group. As expected, the CETP inhibitor had little effect on LDL-cholesterol levels. At the interim analysis (median follow-up 31 months), the data and safety monitoring board recommended termination of the trial for futility. The cumulative event rate was essentially the same in the placebo and dalcetrapib groups (8.0% vs 8.3%, respectively), and the drug had no effect on any component of the primary end point (a composite of major adverse cardiovascular events) or on mortality. Furthermore, modest but significant ($P<0.001$) increases in systolic blood pressure (0.6 mmHg) and C-reactive protein level (0.2 mg/l) were noted with dalcetrapib when compared with placebo.[6] Thus, this new trial did not provide evidence that selectively increasing HDL-cholesterol levels (at least through CETP inhibition) reduces the risk of recurrent cardiovascular events.

Another approach fuelling the HDL-cholesterol target debate relies on Mendelian randomization analyses. If a direct relationship between HDL cholesterol and cardiovascular outcomes exists, genetic variants associated with altered HDL-cholesterol levels, but that have no influence on other lipid or nonlipid risk factors, should be related to cardiovascular disease. Voight *et al.* examined a single nucleotide polymorphism in the endothelial lipase gene (*LIPG* Asn396Ser) in 20 studies that included a total of 20,913 patients with myocardial infarction (MI) and 95,407 healthy control individuals.[7] Their findings were published in May 2012. Carriers of the *LIPG* 396Ser allele (2.6% frequency) had higher HDL-cholesterol levels (+0.14 mmol/l) than noncarriers, but showed no difference in levels of other lipid or nonlipid risk factors for MI. This finding enabled the investigators to test the hypothesis that lifetime exposure to a high HDL-cholesterol level might be cardioprotective. However, the increased HDL-cholesterol level linked with the 396Ser allele was not associated with a reduced risk of MI. This finding differed markedly from the 13% risk reduction (OR 0.87, 95% CI 0.84-0.91) that the investigators predicted on the basis of observational epidemiology studies.[7] In addition, Voight and colleagues calculated a genetic risk score, which included 14 single nucleotide polymorphisms exclusively associated with increased HDL-cholesterol level and, again, found no relationship between these alleles and cardiovascular events.[7] These results indicate that genetic variation associated with lifetime increased HDL-cholesterol level, but not with other cardiovascular risk factors, does not seem to be associated with a reduced risk of MI.

The results of this interesting analysis do not, however, provide the final answer as to whether or not HDL should be targeted to optimize protection against cardiovascular events. At this stage, one point can be made with certainty—not all approaches that increase the HDL-cholesterol level will translate into clinical benefits. For example, CETP inhibitors that do not also reduce LDL-cholesterol levels are probably useless. Whether more-potent CETP inhibitors, such as anacetrapib and evacetrapib that markedly increase HDL-cholesterol levels and decrease levels of non-HDL cholesterol, reduce atherosclerosis and related cardiovascular events is currently being tested in large trials.

In this context, we need to keep in mind that HDL cholesterol is only one feature of HDL. These lipoprotein particles have numerous properties that contribute to slow the development of atherosclerosis, but are not captured by the measurement of HDL-cholesterol concentration. HDL cholesterol only reflects the cholesterol content of the HDL fraction that is most often isolated by precipitation techniques. Numerous techniques are now available to assess the various other properties of HDL, including composition, size, migration on 2D gels, metabolomics, and the capacity to promote cholesterol efflux and act as antioxidants (Figure 1a). Thus, an expanded panel of HDL 'metrics' should be studied to further our understanding of the features of HDL that could be targeted for cardioprotection.

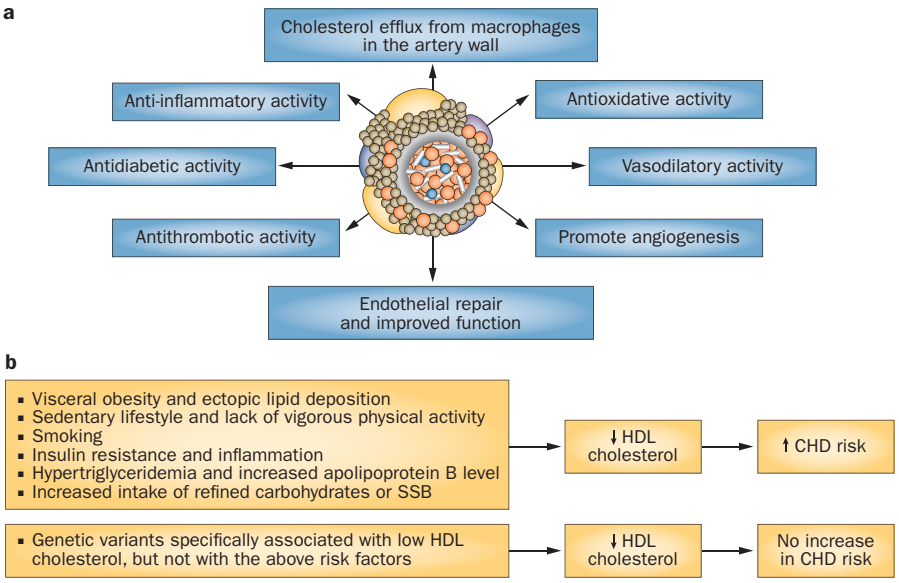

Figure 1 | Mechanisms linking HDL to cardiovascular disease. Despite **a** | properties that are potentially protective against the development of atherosclerosis, **b** | a low HDL-cholesterol level that is not accompanied by other lipid or nonlipid risk factors does not seems to be associated with an increased risk of coronary heart disease. Abbreviations: CHD, coronary heart disease; SSB, sugar-sweetened beverages.

Key advances

- Selectively increasing the HDL-cholesterol level with a cholesteryl ester transfer protein inhibitor (dalcetrapib) that did not decrease the LDL-cholesterol level did not reduce the risk of recurrent cardiovascular events[6]
- Genetic variants that are associated with altered HDL-cholesterol levels, but that have no additional influence on other lipid or nonlipid risk factors, were found to be unrelated to cardiovascular events[7]
- HDL particle number, assessed by NMR, was consistently found in two studies to be one feature of HDL that predicts cardiovascular events[8,9]

Analyses of MESA[8] and the MRC/BHF Heart Protection Study,[9] both published in 2012, suggested that the association between HDL-cholesterol level and cardiovascular outcomes was largely attenuated after control for other features of the lipoprotein profile. On the other hand, HDL particle number assessed by NMR was independently associated with cardiovascular outcomes. These findings suggest that some properties of HDL might provide information beyond HDL cholesterol itself, which seems to be largely a marker of other lipid and nonlipid risk factors. We have previously suggested that low HDL-cholesterol level is usually accompanied by abdominal obesity, lack of physical activity and sedentary lifestyle, insulin resistance, elevated triglyceride and apolipoprotein B levels, and by a state of low chronic inflammation (Figure 1b), key correlates that are predictive of increased cardiovascular disease risk.[10] Therefore, low HDL-cholesterol level in isolation is a rare disorder. Such key correlates of low HDL-cholesterol level might explain why findings from observational epidemiology studies are discordant with results from clinical trials and Mendelian randomization studies. From population health and clinical stand points, although clearly associated with a low risk of coronary heart disease, a high HDL-cholesterol level is largely the consequence of healthy lifestyle habits, a low level of visceral adipose tissue and ectopic fat, and high insulin sensitivity.

Although studies on HDL published in 2012 have generated 'more of the same', such literature adds to the debate. Additional preclinical and clinical data will be required to answer the key question—is HDL cholesterol only an HDL-related marker of cardiovascular risk, or could other features of HDL be targeted as well? Meanwhile, to generate high physiological levels of functional HDL particles, we should watch our waistlines and stay physically active.

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BRAIN CANCER IN 2012

Molecular characterization leads the way

Roger Stupp and Monika E. Hegi

In 2012, advances in molecular profiling of primary brain tumours allowed identification of subgroups of glioma and medulloblastoma that were associated with distinct prognoses and predicted treatment response. Adjuvant chemotherapy is now established for 1p/19q co-deleted anaplastic oligodendrogliomas, and may be the preferred treatment in elderly patients with glioblastoma with a methylated *MGMT* promoter.

Stupp, R. & Hegi, M. E. *Nat. Rev. Clin. Oncol.* **10**, 69–70 (2013); published online 8 January 2013; doi:10.1038/nrclinonc.2012.240

Research and treatment of primary brain tumours has unique challenges, such as access to tumour tissue, as only small quantities of mostly heterogeneous tumours are available for study. Despite these obstacles, important research focused on tumour biology has established definitively the value of molecular markers in the management of malignant glioma. Co-deletion of 1p/19q and methylation of the methylguanine methyltransferase (*MGMT)* gene promoter allows selection of the optimal treatment strategy for patients with glioblastoma and anaplastic oligodendroglial tumours.

Two randomized trials published in 2012 evaluated the role of temozolomide chemotherapy versus radiotherapy in the management of elderly patients (>60–70 years) with malignant glioma.[1,2] In both trials, tumour tissue was collected, and the methylation status of *MGMT* was assessed. MGMT is a DNA repair enzyme that blunts the cytotoxic effect of alkylating agents such as temozolomide. Overall, median survival is unsatisfactory (6–10 months) with no substantial advantage of chemotherapy over radiotherapy. However, both trials demonstrated that patients with an epigenetically silenced *MGMT* gene fared better when treated with temozolomide, whereas patients with an unmethylated *MGMT* promoter had a longer survival with initial radiotherapy. These results corroborate earlier findings regarding the value of *MGMT* methylation as a predictive marker for the benefit of temozolomide chemotherapy in patients with glioblastoma.

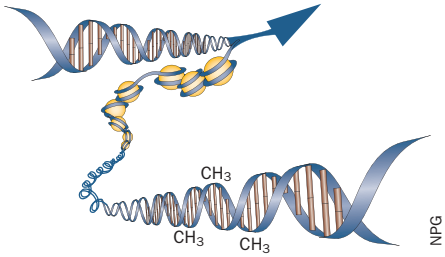
Oligodendroglial tumours are a distinct entity characterized by a prolonged natural history and an exquisite response to chemotherapy or radiotherapy. So far, however, adjuvant chemotherapy has failed to improve survival in unselected patients with anaplastic oligodendroglial tumours. Long-term follow-up in 2012 of two randomized trials that were initiated almost 18 years ago and that investigated adjuvant PCV (procarbazine, lomustine, vincristine) chemotherapy before or after radiotherapy has now shown an improvement in overall survival with adjuvant treatment.[3] Molecular tumour analysis demonstrated that only patients with a combined deletion of chromosomes 1p and 19q, mediated by a translocation [t(1;19)(q10;p10)], benefit from the early introduction of chemotherapy.[3]

Key advances

- Molecular marker analyses should be part of the standard diagnostic workup in all patients with glioma;[1–3,7] prognostic and predictive markers have direct implications in routine clinical decision making
- Long-term follow-up for >11 years demonstrated that adjuvant chemotherapy prolongs survival in patients with anaplastic oligodendroglioma harbouring a co-deletion on chromosomes 1p/19q[3]
- Systematic profiling of medulloblastoma has led to a clinically relevant molecular classification and prognostication,[8,9] allowing for risk-adapted treatment strategies and novel therapeutic targets

Management of low-grade (grade II) glioma remains controversial. Radiotherapy at initial diagnosis does not increase overall survival compared with radiation given at tumour progression, and the role of chemotherapy is unclear. In 2012, the RTOG9802 randomized Intergroup study evaluated radiotherapy with or without adjuvant PCV-chemotherapy in high-risk patients with low-grade (grade II) glioma.[4] Between 1998 and 2002, 251 patients were randomly assigned to receive radiotherapy alone or radiotherapy followed by adjuvant chemoradiation. Progression-free survival improved with adjuvant chemotherapy; however, the primary end point was not met—no overall survival benefit was demonstrated. Nevertheless, when patients with worst prognosis (16%, defined as patients dying within 2 years and thus likely harbouring an unrecognized higher grade tumour) were excluded from the analysis, only those patients who remained alive after 2 years seemed to derive a benefit from adjuvant chemotherapy.[4] Molecular tumour data are not yet available, and the first results of the EORTC-led Intergroup trial (EORTC 22033-26033/NCIC CE.5) comparing dose-intense temozolomide chemotherapy (21 days of a 28-day cycle) versus radiotherapy are expected in 2013. In this trial, tumour tissue has been prospectively analysed for molecular markers, and patients have been stratified for the presence of the 1p deletion, a known favourable prognostic factor.

Virtually all patients with malignant glioma will experience tumour recurrence. Inhibition of VEGF has resulted in impressive radiological responses with an unprecedented rapid decrease in contrast enhancement and reduction in peritumoural oedema. However, whether this temporary restoration of the blood–brain barrier translates into prolonged survival and a benefit to patients remains the subject of vivid controversy. The anti-VEGF antibody bevacizumab has finally been investigated in a randomized phase III trial in patients with newly diagnosed glioblastoma. Initial results of the AvaGlio study were presented in abstract form.[5] As expected, progression-free survival was improved; however, this did not translate into prolonged overall survival



at the first interim analysis. Thus, the true value of bevacizumab remains unclear, and although it seems to be of benefit in selected patients with recurrent glioma, administration of bevacizumab early in the disease course may not be justified. Unfortunately, biomarkers for the optimal use of bevacizumab have not been identified yet. Longer term follow-up and the results of a similarly designed RTOG study are awaited (results are expected for ASCO 2013).

An entirely novel cancer treatment modality with alternating electrical fields—the so-called tumour treatment fields (TTF)—is being investigated in newly diagnosed and recurrent glioblastoma. Experimental models have shown that rapidly alternating electrical fields interfere with cell division by disturbing mitotic spindle formation and lead to dielectrophoretic movement of intracellular macromolecules and organelles, resulting in cell death. A randomized controlled phase III trial was conducted in heavily pretreated patients with recurrent glioblastoma. Patients were randomly assigned to either physician's best choice of chemotherapy (control) or TTF without any chemotherapy.[6] This trial demonstrated the feasibility of this approach, with a slightly higher response rate observed for patients treated with TTF (14% versus 10%, $P = 0.2$), and fewer severe adverse events in the TTF arm. However, no significant difference in overall survival was noted (6.6 months versus 6.0 months). On the basis of the observed objective responses with this modality alone, and synergy with chemotherapy in preclinical models, NovoTTF (NovoCure Ltd, Haifa, Israel) is currently under investigation in a phase III clinical trial in patients with newly diagnosed glioblastoma, as an addition to adjuvant or maintenance temozolomide chemotherapy.

Mutations of the isocitrate dehydrogenase (*IDH*) genes have been identified as an early event in gliomagenesis. These mutations are a typical feature of low-grade glioma, secondary (transformed) higher grade glioma and glioblastoma, and are associated with a more-favourable prognosis. Ongoing research is aimed at targeting this enzyme for therapeutic purposes. *IDH* mutations result in a neomorphic reaction yielding the oncometabolite 2-hydroxyglutarate (2HG). Subsequent accumulation of this metabolite in the tumour tissue interferes with the epigenetic machinery and induces a CpG island methylator phenotype. Recent advances have shown that magnetic resonance spectroscopy (MRS) is able to detect 2HG in a non-invasive manner.[7] MRS may provide an easier molecular characterization and quantification of glioma, and a way to evaluate the response to therapy early in the disease course, because typical morphological response often requires months, and MRI evaluation is often ambiguous in these slow-growing tumours. Growth acceleration and malignant transformation might also be identified earlier than with conventional imaging techniques.

The importance of molecular tumour characterization is also well illustrated by recent developments in medulloblastoma. Systematic molecular profiling has allowed identification of at least four distinct molecularly defined subgroups that also correspond to some clinical features and outcome.[8] Whole-genome sequencing and analysis of copy-number aberrations enables further refinement of the disease subgroups and identification of relevant new genes or epigenetic alterations.[9] New findings were published simultaneously in four publications in the August issue of *Nature*. Importantly, these insights into disease pathogenesis open avenues for novel treatments. About 30% of patients with medulloblastomas have activation of the sonic hedgehog (SHH) signalling pathway, and small-molecule inhibitors of Smoothened, a co-activator of SHH, are being tested in clinical trials. One such agent, vismodegib is currently being tested in patients with medulloblastoma. In 10% of patients with medulloblastoma, the Wnt signalling pathway is activated and is associated with a favourable prognosis.[8] This allows de-escalation of treatment intensity while maintaining curative intent. Despite the rarity of medulloblastoma, the strict collection of fresh tumour tissue over many years, and the exceptional international collaborations have provided unprecedented insights into this disease that will have consequences on clinical management.

The past year illustrates how molecular tumour characterization has grown beyond the exclusive research setting. An increasing number of markers have been validated for their prognostic and predictive power, and have demonstrated great value for everyday clinical decision making. Furthermore, molecular profiling has improved our understanding of pathogenesis and opens novel avenues for therapeutic approaches. Although therapeutic progress has been rather modest, anecdotal evidence suggests that some patients may derive a true benefit from dose intensification, antiangiogenic agents and novel treatment modalities. Better tumour characterization will allow identification of patients most susceptible who are likely to benefit from a specific treatment—personalized strategies are on the horizon.

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COLORECTAL CANCER IN 2012

Revisiting landmark trials and identifying new therapies

Christina Wu and Richard M. Goldberg

In the past year, long-term follow-up of trials have confirmed and disproved paradigms in the treatment of colorectal cancer, and identified a chemoprevention agent. In metastatic disease, chemotherapy in unresected primary tumours was studied, and randomized phase III trials introduced new therapy options. Molecular characterization of colon and rectal tumours offers new drug targets.

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Key advances

- Aspirin is effective as chemoprevention for patients with Lynch syndrome[1]
- Regorafenib and aflibercept are new drugs for metastatic colorectal cancer[7,9]
- Bevacizumab use past disease progression provides survival benefit[8]
- New targetable genes have been discovered in colorectal cancer[10]

Research published during 2012 is changing the way we manage patients with colorectal cancer. Long-term follow-up of clinical trials established the efficacy of aspirin for primary prevention of cancer in individuals with Lynch syndrome; confirmed the efficacy of neoadjuvant chemoradiation in the treatment of rectal cancer; and analysed the benefits of adjuvant oxaliplatin in patients with colon cancer. In the metastatic setting, studies supported the safety of chemotherapy in unresected primary tumours, demonstrated the continued efficacy of bevacizumab after disease progression, and introduced two new FDA-approved drugs, regorafenib and aflibercept.

In the field of colorectal cancer prevention, the results of the CAPP2 trial established aspirin as a chemoprevention agent for patients with Lynch syndrome, who have an 80% lifetime risk for colon cancer.[1] This trial was a considerable advance because it was the first trial of aspirin that had colorectal cancer development as the primary end point. The study randomly assigned 937 patients to aspirin or placebo, and resistant starch or placebo. Initial follow-up at 29 months showed no benefit for either of the interventions, but at 55.7 months, aspirin was shown to reduce cancer incidence, with a hazard ratio (HR) of 0.63 (95% CI 0.35–1.13; $P = 0.12$).[1] Among individuals who took 2 years of aspirin, the HR of developing colorectal cancer was 0.41 (95% CI 0.19–0.86; $P = 0.02$). The optimal dose and duration of aspirin administration remains to be determined. Doses as low as 81 mg/day have been associated with reduced polyp formation in high-risk patients unselected for Lynch syndrome and the risk of gastritis with chronic aspirin use is dose related. At present, we are recommending daily 650 mg of enteric-coated aspirin indefinitely in patients with Lynch syndrome who have no contraindications to aspirin use.

> “...evaluation of new approaches to optimize outcomes remains a research priority”

Advances in treatment regimens were also made in 2012. In locally advanced rectal cancer, the German CAO/ARO/AIO-94 trial—which initially was reported in 2004—shifted the treatment paradigm from postoperative to preoperative chemoradiation as the standard of care. Patients were randomly assigned to preoperative chemoradiation, surgery, and adjuvant chemotherapy or the same schedule received postoperatively. At a median follow-up of 134 months it was possible to confirm a decreased local recurrence of 6.8% with preoperative therapy versus 10.5% with postoperative therapy (HR = 0.54; 95% CI 0.3–0.9; $P = 0.02$).[2] Based on these data and others, as standard care we recommend preoperative therapy to patients with indications on MRI or endorectal ultrasound suggesting transmural or node-positive disease and primary surgery for earlier stage patients who may be spared radiation and its potential for long-term side effects. However, the overall survival of 40% and disease-free survival of 68% were equivalent in both arms of the CAO/ARO/AIO-94 trial after this long follow-up, which highlights the need for more approaches to improve survival going forward.

Treatment advances for patients with colon cancer came via the MOSAIC trial; investigators reported a post-hoc analysis limited to stage II patients and another analysis of stage II and III elderly patients who participated in this colon cancer adjuvant trial.[3] Patients with stage II colon cancer who were either high-risk (defined by T4 staging, tumour perforation, bowel obstruction, poorly differentiated tumour, venous invasion, or fewer than 10 lymph nodes examined) or low-risk experienced only marginal benefit from the addition of oxaliplatin to 5-fluorouracil (5-FU). In addition, elderly patients between 70 and 75 years of age with stage II or III cancer did not achieve a survival benefit from the addition of oxaliplatin. These findings were consistent with results from a retrospective study in which 5,489 patients who were 75 years and older and had stage III colon cancer experienced a survival advantage with adjuvant chemotherapy, but the addition of oxaliplatin offered little incremental benefit.[4] These findings suggest that in both stage II and elderly patients, much of the benefit of adjuvant therapy can be achieved using 5-FU alone and the addition of oxaliplatin, with its potential for long-term sensory neuropathy, should be considered on a case-by-case basis. With 30% of patients relapsing despite optimal surgery and adjuvant therapy, evaluation of new approaches to optimize outcomes remains a research priority.

For synchronous metastatic colorectal cancer, debate flourishes regarding whether to resect asymptomatic primary tumours to avoid bleeding, obstruction, or perforation or to treat with chemotherapy and spare patients from surgery. It seems that this question has at least in part been answered by the results from a prospective analysis of 233 patients, where 93% never required surgery, and bevacizumab (an anti-VEGF-A antibody) use was not associated with increased surgery rates.[5] In 2012, the NSABP C-10 phase II study went further and assessed 5-FU, leucovorin, and oxaliplatin (FOLFOX) and bevacizumab in 86 patients with asymptomatic primary tumours with a median follow-up of 20.7 months. The major morbidity rate related to the primary tumour was 16.3% (95% CI 7.6–25.1%), and the median overall

survival was 19.9 months.[6] This relatively small study demonstrated that chemotherapy with bevacizumab can be safely administered in patients with asymptomatic primary tumours. These two studies support management of patients who present with metastatic disease with initial chemotherapy including angiogenesis targeted agents without surgery, but with vigilant follow-up to permit early intervention in the case of local complications.

> "Translational research has also been driving forward clinical possibilities throughout 2012"

Keeping with the targeting of angiogenesis in patients with metastatic colorectal cancer (Table 1), the VELOUR trial examined aflibercept, a recombinant protein of VEGFR-1 and VEGFR-2 that targets ligands VEGF-A, VEGF-B, and PGF.[7] This phase III study randomly assigned 1,227 patients who had previously received oxaliplatin-based treatment to 5-FU and irinotecan with or without aflibercept. The addition of aflibercept improved median survival from 12.06 months to 13.50 months (HR = 0.817; 95% CI 0.713–0.937; $P = 0.0032$). Notably, among the 373 patients with prior bevacizumab exposure, the survival benefit was less than that observed in bevacizumab-naive patients. Aflibercept led to a higher incidence of anti-VEGF and chemotherapy-related adverse effects. As shown in prior studies, bevacizumab improves survival when combined with first-line chemotherapy regimens, and the phase III TML study investigated the potential for ongoing benefit with continued administration in the second-line setting.[8] In 820 patients randomly assigned to chemotherapy with or without bevacizumab, there was improved overall survival in the bevacizumab arm; 11.2 months versus 9.8 months (HR = 0.81; 95% CI 0.59–0.94; $P = 0.0062$). Anti-VEGF related adverse events were not increased with continuation of bevacizumab therapy. The VELOUR and TML studies both offer novel anti-VEGF treatment options for patients in this setting. In addition, regorafenib, an oral multikinase inhibitor, was studied in 760 patients who were refractory to standard therapies. The patients were randomly assigned to regorafenib and best supportive care (BSC) or placebo and BSC in the CORRECT trial. After 566 events had occurred, regorafenib therapy was associated with improved overall survival (6.5 months versus 5 months; HR = 0.79; 95% CI 0.66–0.94; $P = 0.0038$).[9] The most-frequent adverse events were hand–foot syndrome, fatigue, diarrhoea, hyperbilirubinemia, and hypertension. Taken together, these targeted agents offer treatment options for patients who have failed on previous lines of therapy.

Translational research has also been driving forward clinical possibilities throughout 2012. Molecular characterization of individual tumour types has the potential to elucidate the clinical relevance of the multiple pathways that drive carcinogenesis. The Cancer Genome Atlas Network profiled 276 human colon and rectal cancers noting that both share similar patterns of genomic alterations.[10] Known gastrointestinal-cancer associated pathways, such as the RAS and PI3K pathways, were found to have targetable mutations. However, dysregulated Wnt signalling and β-catenin pathway alterations were identified and offer new areas to direct drug development.

Over the past year, our knowledge of the biology behind the prevention of and therapy for colorectal cancer has improved, with confirmation of the efficacy of established therapies, new findings in aspirin chemoprevention, and the lack of benefit of adjuvant oxaliplatin in certain subgroups. Asymptomatic primary tumours can be safely treated with FOLFOX and bevacizumab in metastatic cancers, and bevacizumab can be used beyond disease progression. Exciting new drugs, such as regorafenib and aflibercept, have been added to our arsenal, and as we continue to molecularly profile colorectal cancer, we will be able to better target oncogenic pathways.

Table 1 | Phase III clinical trials for metastatic colorectal cancer

Trial, study arm	Regimen	Overall survival		
		Months	HR	*P* value
TML, $n = 820$	Bevacizumab and 5-FU-based CT vs 5-FU-based CT[8]	11.2 vs 9.8	HR 0.81; 95% CI 0.59–0.94	$P = 0.0062$
VELOUR, $n = 1,227$	Aflibercept and FOLFIRI vs FOLFIRI[7]	13.50 vs 12.06	HR 0.817; 95% CI 0.713–0.937	$P = 0.0032$
CORRECT, $n = 760$	Regorafenib and BSC vs BSC alone[9]	6.5 vs 5.0	HR 0.79; 95% CI 0.66–0.94	$P = 0.0038$

Abbreviations: 5-FU, 5-fluorouracil; BSC, best supportive care; CT, chemotherapy; HR, hazard ratio.

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Competing interests
R. M. Goldberg declares associations with the following companies: Bayer, Eli Lilly, Fresenius Kabi, Sanofi. See the article online for full details of the relationships. C. Wu declares no competing interests.

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GASTRIC CANCER IN 2012

Defining treatment standards and novel insights into disease biology

Elizabeth C. Smyth and David Cunningham

Gastric cancer is a heterogeneous disease with almost one million new cases occurring annually worldwide. The year 2012 saw important successes and failures in gastric cancer treatment, and also novel insights into the molecular characterization of this disease, which may lead to the development of more-effective targeted therapies.

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The incidence of gastro-oesophageal junction cancer continues to increase worldwide. Current approaches for patients with operable disease include perioperative chemotherapy followed by surgery; surgery followed by postoperative chemoradiation; or surgery alone for patients with early stage disease. In 2012, the final results of the CROSS study, showed that chemoradiation followed by surgical resection for the treatment of the gastro-oesophageal junction subset of gastric cancers was significantly superior to surgery alone.[1] This study compared the use of surgery alone with a combination of weekly carboplatin and paclitaxel used in conjunction with radiotherapy followed by surgery. Patients were required to have T1N1 or T2–3 N0–1 staged cancers; 64% of patients were lymph-node positive on endoscopic ultrasound before starting treatment. Most (75%) of the 368 patients enrolled in the study had an adenocarcinoma diagnosis; 58% of patients had distal oesophageal tumours and 24% of patients had tumours located at the gastro-oesophageal junction (excluding proximal gastric tumours with minimal invasion of the oesophagus). Overall survival in the chemoradiation group was 49 months compared with 24 months in the surgery-alone group (Table 1).[1] However, the treatment effect adjusted for baseline characteristics was significant only for patients with squamous-cell carcinoma (SCC) and not for adenocarcinoma, with adjusted hazard ratios (HR) of 0.422 (95% CI 0.226–0.788, $P = 0.007$) and 0.741 (95% CI 0.536–1.024, $P = 0.07$), respectively. Additionally, a non-significant adjusted HR was demonstrated for patients with lymph-node-positive cancers (HR 0.807; 95% CI 0.576–1.130, $P = 0.21$).[1] Although this study demonstrates improved survival outcomes for the arm receiving chemoradiation followed by surgery, these results may be driven partly by the significant results in the more-radiosensitive population with SCC and in those patients with node-negative cancers. However, due to the increased response rate seen with the addition of radiotherapy (23% pathological complete response [pCR] in adenocarcinoma and 49% in SCC), this regimen remains a reasonable option for patients with adenocarcinoma with more-locally advanced disease who may be at risk of a positive resection margin. Unanswered questions include whether chemoradiation would be superior to a more-current control arm—such as perioperative chemotherapy—and whether the addition of surgery to the combination regimen for patients with SCC is necessary for patients with a sustained pCR.

In Asia, adjuvant chemotherapy with the oral fluoropyrimidine S-1 is associated with an overall survival benefit and is routinely offered to patients after D2 surgical resection.[2] The CLASSIC study[3] examined the use of adjuvant XELOX chemotherapy (oxaliplatin and capecitabine) compared with surgery in Asian patients with gastric cancer ($n = 1{,}035$) who had undergone D2 surgical resection.[3] Gastro-oesophageal junction tumours were rare (<3%), and most tumours (>90%) were node positive (mean number of lymph nodes resected was >40). A significant benefit in 3-year disease-free survival (DFS) was seen for the chemotherapy arm of the study (74% in the group receiving adjuvant chemotherapy and surgery, and 59% in the surgery-alone group, which is comparable to the DFS seen with S-1 monotherapy). Overall survival data are not yet mature. Toxicity was significant (56% grade 3–4 toxic effects in the experimental arm), leading to dose modifications in 90% of patients; only 67% of patients completed chemotherapy. It is debatable whether these results can be extended to non-Asian populations. Although D2 resection is now recommended in Europe and the USA, important differences with the Asian population remain, such as biological heterogeneity and the necessity to downstage Western patients before surgery owing to their more-advanced stage at presentation.

Currently, standard treatment for patients with advanced-stage gastric cancer and a good performance status is a platinum and fluoropyrimidine doublet with the optional addition of an anthracycline or taxane for

Table 1 | Key trials in gastric cancer in 2012

Study	Treatment arms	OS	Treatment effect	Comments
CROSS[1] (neoadjuvant)	Surgery vs neoadjuvant CRT	24 49	HR 0.657 (95% CI 0.495–0.87) $P=0.003$	NS for adenocarcinoma, node-positive patients
CLASSIC[3] (neoadjuvant)	Surgery vs adjuvant XELOX	59%* 74%*	HR 0.56 (95% CI 0.44–0.72) $P<0{\cdot}0001$	Results comparable to S1 monotherapy; 67% of patients completed treatment; generalizability questionable
REAL3[6] (advanced-stage)	EOC vs mEOC–P	11.3 8.8	HR 1.37 (95% CI 1.07–1.76) $P=0.013$	1st-line therapy; decreased dose of oxaliplatin and capecitabine in mEOC–P arm; recruitment terminated early
EXPAND[7] (advanced-stage)	CX vs CX + cetuximab	10.7 9.4	HR 1.004 (95% CI 0.866–1.165) $P=0.9547$	1st-line therapy; similar chemotherapy intensity in each treatment arm
Kang *et al.*[4] (advanced-stage)	BSC vs docetaxel or irinotecan	3.8 5.3	HR 0.657 (95% CI 0.48–0.891) $P=0.007$	Patients receiving 2nd-line and 3rd-line therapy were included
GRANITE-1[8] (advanced-stage)	BSC + placebo vs everolimus	4.3 5.4	HR 0.90 (95% CI 0.75–1.08) $P=0.1244$	Patients receiving 2nd-line and 3rd-line therapy were included

*3-year DFS. Abbreviations: BSC, best supportive care; CRT, chemoradiotherapy; CX, cisplatin and capecitabine; DFS, disease-free survival; EOC, epirubicin, oxaliplatin and capecitabine; HR, hazard ratio; mEOC, modified EOC; mEOC–P, mEOC + panitumumab; NS, non-significant; OS, overall survival in months; XELOX, oxaliplatin and capecitabine.

Key advances

- Chemoradiation followed by surgery is superior to surgery alone for patients with resectable oesophageal and gastro-oesophageal carcinoma[1]
- Anti-EGFR antibody therapy added to chemotherapy in non-molecularly selected gastric cancer populations did not improve survival[6,7]
- The MET–HGF axis seems to be a promising therapeutic target[10]

selected patients; however, no previous randomized trials have demonstrated the efficacy of second-line chemotherapy. This year, for the first time, Kang *et al.*[4] demonstrated, a survival benefit associated with salvage chemotherapy following disease progression on first-line or second-line treatment in patients with advanced-stage gastric cancer.

To date, with the exception of trastuzumab in HER2-positive patients, targeted therapies have been disappointing in non-molecularly selected patient populations with gastric cancer.[5] Although phase II studies of anti-EGFR therapy in advanced-stage gastric cancer were promising, negative results from two large first-line phase III randomized trials that compared the use of standard first-line chemotherapy plus or minus an anti-EGFR antibody were presented in 2012.[6–8] The REAL3 study ($n = 553$) compared the addition of panitumumab (P) to EOC (epirubicin, oxaliplatin and capecitabine) chemotherapy (Table 1).[6] Median overall survival was significantly worse in the EOC-P combination arm (8.8 months versus 11.3 months), and enrolment was terminated early. After a phase I safety assessment in the EOC-P arm, combination doses of oxaliplatin and capecitabine had been reduced compared with the standard regimen. This adjustment may, in part, explain the inferior results seen in the experimental arm. Initial biomarker analysis had revealed that mutations in *KRAS* was a negative prognostic indicator (HR 2.1, 95% CI 1.10–4.05, $P = 0.025$); however, the rate of this mutation was too low (<5%) to be used to predict accurately the response to anti-EGFR therapy. Similarly, the EXPAND study randomly assigned 904 previously untreated patients with advanced-stage gastro-oesophageal cancer to cisplatin and capecitabine chemotherapy with or without cetuximab.[7] In this trial, exposure to chemotherapy was similar in both treatment arms. However, no significant differences were seen in response rate, progression-free survival or overall survival between the two regimens. Finally, the third large randomized phase III trial with negative results presented in 2012 was the GRANITE-1 study.[8] This study compared best supportive care in combination with either everolimus or placebo for patients with advanced-stage gastric cancer and disease progression after one or two lines of systemic therapy.[7] No significant difference was demonstrated in overall survival between the treatment arms (5.4 months versus 4.3 months for the everolimus and placebo groups, respectively). Together, these disappointing results highlight the need to design clinical trials to target advanced gastro-oesophageal cancer appropriately.

In contrast to melanoma, colorectal cancer, and non-small-cell lung carcinoma, rates of activating mutations in gastric cancer are low. However, assessments of gene copy-number alterations have revealed distinct molecular phenotypes that may be amenable to treatment with novel targeted therapies. Deng *et al.*[9] characterized 233 gastric tumours using high-resolution single nucleotide polymorphism arrays and revealed amplifications of several tyrosine kinases (RTK)—such as *FGFR2, EGFR, HER2, KRAS* and *MET*—in up to 37% of gastric cancers, results that have subsequently been confirmed by other investigators. Notably, a subgroup analysis of a phase II randomized study using rilotumumab—a monoclonal antibody targeting the MET–HGF axis—demonstrated superior overall survival in patients with high levels of MET expression when treated with epirubicin, cisplatin and capecitabine (ECX) in combination with rilotumumab in the first-line setting than with ECX alone (11.1 versus 5.7 months; HR = 0.29, 95% CI 0.11–0.76, $P = 0.012$).[10] Multiple late-stage trials using anti-FGFR and anti-MET targeted therapies are ongoing, and it is hoped that targeting patients with tumours that are driven by amplification or overexpression of these RTKs will be more fruitful in the future than a non-molecularly selected approach.

The past year highlighted the diversity of tumour biology and therapeutic approaches in gastric cancer. No consensus exists regarding the optimal perioperative treatment of resectable gastric or gastro-oesophageal cancer; however, several reasonable evidence-based treatment options are available for such patients. The use of targeted therapies in unselected populations has not resulted in improvements in overall survival, but emerging evidence suggests that there are distinct subsets of patients who may benefit from these agents. Addressing this heterogeneity within the context of global clinical trials is challenging; thoughtful and collaborative design of future clinical trials and translational research programmes will be essential in order to continue to improve outcomes for patients with gastric cancer.

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D. Cunningham declares associations with the following companies: Amgen, AstraZeneca, Celgene, Merck-Serono, Roche, Sanofi. See the article online for full details of the relationships. E. C. Smyth declares no competing interests.

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BREAST CANCER IN 2012

New drugs, new knowledge, new targets

Mariana Chavez-MacGregor and Ana Maria Gonzalez-Angulo

In 2012, we increased our knowledge of the molecular portrait of breast cancer. The BOLERO-2 and CLEOPATRA trials led to the approval of everolimus and pertuzumab; and the EMILIA trial will likely result in the approval of T-DM1. Some of these findings represent a paradigm shift in the way we think about the biology and management of breast cancer.

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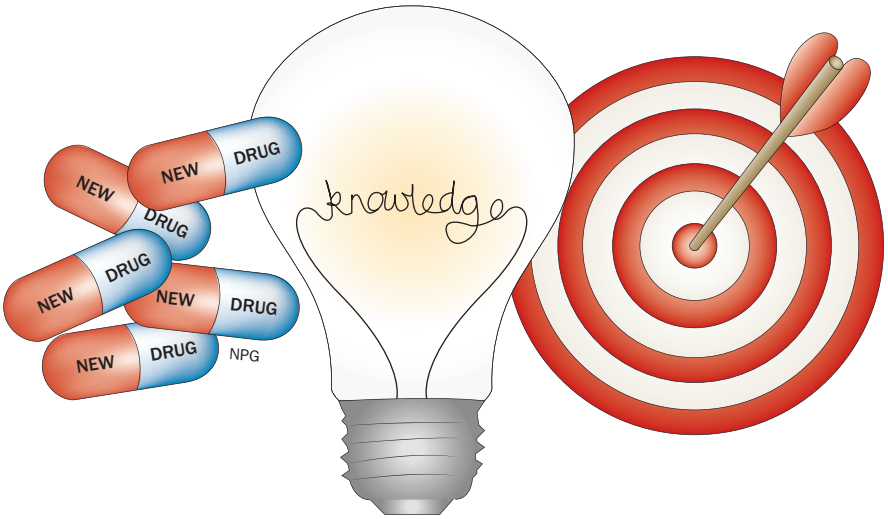
For many years we have known that breast cancer is a heterogeneous disease.[1] Categorizing tumours in different subgroups has important prognostic and clinical implications in our daily practice. The Cancer Genome Atlas Network, as part of an extraordinary collaboration, evaluated a set of breast tumours using six different technologies at the DNA, RNA and protein levels to identify subtype-specific molecular aberrations that could help us understand breast cancer biology, as well as to identify therapeutic targets.[2] The results from the study confirmed the existence of four main breast cancer subtypes (luminal A, luminal B, basal-like and HER2-enriched), each of them molecularly heterogeneous. The luminal (or oestrogen receptor-positive [ER+]) tumours are the most heterogeneous in terms of gene expression and mutational spectrum. A dominant feature among them is the expression of a luminal gene signature (*ESR1*, *GATA3*, *FOXA1*, *XBP1*, and *MYB*). These tumours also have a high rate of *PIK3CA* mutations (45% in luminal A and 29% in luminal B cancers). Frequent mutations were also found in the *MAP3K1* and *MAP2K4* genes, which are important components in the JNK pathway. Among basal-like tumours, a high frequency of *TP53* mutations (80%) was observed, followed by *PIK3CA* mutations (9%). The analysis emphasized the high genomic instability of these subtypes and hyperactivation of *FOXM1* as a transcriptional driver. Other abnormalities include *ATM* mutations, *RB1* loss, *CCNE1* amplification and *BRCA1* and *BRCA2* inactivation (20% germline or somatic mutation). Among the HER2-enriched tumours, two subtypes were identified with 302 genes differentially expressed among them. One group had a high expression of *FGFR4*, *EGFR*, *HER2*, and genes within the *HER2* amplicon. The other subgroup showed high expression of a luminal cluster of genes including *GATA3*, *BCL2* and *ESR1*. HER2-enriched tumours had a high frequency of *PIK3CA* mutations (39%) and a lower frequency of *PTEN* and *PIK3R1* mutations. Other possible druggable targets include variants of the HER family, such as *HER2* and *HER3* mutations.[2]

An example of the clinical implications of these findings is the development of different anti-HER2 therapies. Pertuzumab is a monoclonal antibody that prevents HER2 from dimerizing with other ligand-activated HER2 receptors, particularly HER3. Double receptor blockade has resulted in greater antitumour activity and the combination of trastuzumab (an anti-HER2 antibody) and pertuzumab in early phase studies proved to be efficacious.[3] Building on these data, the phase III CLEOPATRA (Clinical Evaluation of Pertuzumab and Trastuzumab) trial randomly assigned 808 patients with HER2-positive metastatic breast cancer to receive pertuzumab, trastuzumab and docetaxel or trastuzumab, docetaxel and placebo as first-line therapy.[4] Patients treated with pertuzumab had a median progression-free survival (PFS) of 18.5 months compared to 12.4 months in the placebo arm (hazard ratio [HR] = 0.62; 95% CI 0.51–0.75; P <0.001). This PFS benefit was seen among all predefined subgroups, and in patients previously treated with trastuzumab (HR = 0.62; 95% CI 0.35–1.07). The interim analysis for overall survival showed a trend towards a survival benefit for the triple combination. Adverse event occurrences were similar among treatment arms; however, there were more episodes of diarrhoea, rash, mucosal inflammation, febrile neutropenia and dry skin in the pertuzumab arm. These results suggest that the use of therapies that have complementary mechanisms of action results in improved efficacy. On 8 June 2012, the FDA approved the use of pertuzumab in combination with trastuzumab and docetaxel as front-line therapy for patients with HER2-positive metastatic breast cancer.

Trastuzumab emtansine (T-DM1) is another agent that targets HER2 and consists of an antibody–drug conjugate that incorporates trastuzumab with a microtubule inhibitor agent derivative of maytansine, which allows delivery specifically to HER2-overexpressing cells and has been shown to have efficacy in breast cancer.[5,6] The EMILIA study randomly assigned 991 patients with metastatic, unresectable or locally advanced breast cancer who had been previously treated with trastuzumab and a taxane to receive T-DM1 or lapatinib plus capecitabine.[7] Patients treated with T-DM1 had improved PFS compared to patients treated with capecitabine and lapatinib (9.6 months versus 6.4 months; HR = 0.65; 95% CI 0.55–0.77; P <0.001). At the second interim analysis for overall survival at 331 events, T-DM1 significantly increased median overall



Key advances

- The Cancer Genome Atlas Network projects confirmed our knowledge about the heterogeneity of breast cancer and provided knowledge about molecular aberrations that can be used for targeted therapeutics[2]
- Pertuzumab and TDM-1 are novel therapeutics available for the treatment of patients with HER2-positive breast cancer[4,7]
- Everolimus is the first agent to significantly enhance the efficacy of endocrine therapy in patients with metastatic hormone receptor-positive breast cancer[10]

survival (30.9 months versus 25.1 months; HR = 0.68; 95%CI 0.55–0.85; P <0.001). The benefit in PFS and overall survival was seen across subgroups and was independent of the line of therapy. The adverse effect profile of T-DM1 was favourable compared to the capecitabine and lapatinib arm, with only anaemia, thrombocytopenia and elevated liver function tests being more common in the TDM-1 arm. These results will likely lead to the FDA approval of T-DM1. In the era of HER2-targeted therapies, the current challenge is to establish the optimal setting for using each of these agents. Results from upcoming trials should clarify this question.

Among patients with hormone receptor-positive metastatic breast cancer, endocrine therapy resistance represents a considerable challenge. Crosstalk between the ER and the PI3K/Akt/mTOR pathways seems to explain, at least in part, this phenomenon.[8] Everolimus is an mTOR inhibitor that has been evaluated in combination with endocrine agents, and results suggested that its use restores endocrine sensitivity.[9] The BOLERO-2 study randomly assigned (2:1 ratio) 724 postmenopausal patients with hormone receptor-positive breast cancer who had previously been treated with anastrozole or letrozole, to receive everolimus and exemenstane or exemestane and placebo.[10] The median PFS was 6.9 months for the combination arm and 2.8 months for the exemestane and placebo arm, representing an impressive and clinically relevant 57% reduction in the risk of progression (HR = 0.43; 95%CI 0.35–0.54). Subgroup analysis demonstrated the benefit of everolimus in all groups independent of previous treatment. The mature data for overall survival is not yet available, but the interim data show a trend favouring the everolimus-treated patients. Adverse effects were more common in the everolimus group. The most common grade 3 or 4 adverse events were stomatitis, anaemia, and dyspnoea. Despite the differences in toxic effects, no differences in quality of life were observed. Everolimus is the first agent to significantly enhance the efficacy of endocrine therapy in patients with metastatic hormone receptor-positive breast cancer. The use of everolimus in combination with exemestane was approved by the FDA on 20 June 2012 for patients previously treated with anastrozole and letrozole.

In this new era where the care of patients with cancer is moving towards personalized therapy, the improved knowledge of breast cancer biology will help identify new targets. In the future, we will hopefully be able to identify the best way to combine these therapies and to identify the patients that are more likely to benefit from them.

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ACUTE MYELOID LEUKAEMIA IN 2012

En route to improved treatment options

Heiko Becker and Clara D. Bloomfield

Progress was made in major aspects of acute myeloid leukaemia in 2012. Gemtuzumab ozogamicin and decitabine were shown to improve outcomes, relapse after stem-cell transplantation might be prevented by selecting donors according to their *KIR* genotypes, and next-generation sequencing has provided insights into mutational patterns and disease evolution.

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Despite considerable improvements in outcome—particularly in younger patients—acute myeloid leukaemia (AML) remains a fatal disease for most patients. In 2012, a report from the Acute Leukemia French Association (ALFA)[1] is likely to affect the

Table 1 | Outcomes in phase III trials of GO added to induction therapy in patients with previously untreated AML

Study	*n*	Age (median) in years	Treatment	Outcomes (GO versus no GO)		
				CR	RFS	OS
ALFA 0701[1]	278*	50–70 (62)	Induction: daunorubicin and cytarabine with or without three doses of GO (3 mg/m^2) Consolidation: daunorubicin and cytarabine with or without one dose of GO (3 mg/m^2)	81%‡ versus 75% (P=0.25)	2-year: 50.3% versus 22.7% (P=0.0003)	2-year: 53.2% versus 41.9% (P=0.0368)
MRC AML16[2]	1,115§	51–84 (67)	Induction: daunorubicin and cytarabine, or daunorubicin and clofarabine, with or without one dose of GO (3 mg/m^2) Consolidation: none or daunorubicin and cytarabine	62% versus 58% (P=0.14)	3-year: 21% versus 16% (P=0.04)	3-year: 25% versus 20% (P=0.05)
MRC AML15[3]	1,113‖	0–71 (49)	Induction: daunorubicin and cytarabine, or daunorubicin, cytarabine and etoposide, or FLAG-Ida, with or without one dose of GO (3 mg/m^2) Consolidation: cytarabine or MACE/MidAC with or without one dose of GO (3 mg/m^2)	82% versus 83% (P=0.8)	5-year: 39% versus 35% (P=0.09)	5-year: 43% versus 41% (P=0.3)
SWOG S0106[4]	506¶	NR#	Induction: daunorubicin** and cytarabine, with or without one dose of GO (6 mg/m^2) Consolidation‡‡: cytarabine	66% versus 69% (P=NR)	HR 1.00, 95% CI 0.69–1.44 (P=0.5)	Median: 31 months versus 35 months (P=NR)

*Patients with *de novo* AML. ‡Including CRp. §Patients with *de novo* or secondary AML or MDS with >10% bone marrow blasts. ‖Patients with *de novo* or secondary AML. ¶627 patients with *de novo* AML were registered, 506 patients were considered for CR analysis. #Patients eligible for inclusion were adults age 18–60 years. **Daunorubicin dose was 45 mg/m^2 in the GO arm, 60 mg/m^2 in the control arm. ‡‡Patients in CR after consolidation were randomized to three doses of GO (5 mg/m^2) or none. Abbreviations: ALFA, Acute Leukemia French Association; AML, acute myeloid leukaemia; CR, complete remission; CRp, complete remission with incomplete platelet recovery; FLAG-Ida, fludarabine, cytarabine, granulocyte colony-stimulating factor and idarubicin; GO, gemtuzumab ozogamicin; MACE/MidAC, amsacrine, cytarabine and etoposide followed by mitoxantrone and cytarabine; MRC, UK Medical Research Council; NR, not reported; OS, overall survival; RFS, relapse-free survival; SWOG, Southwest Oncology Group.

treatment of patients with AML. In this randomized phase III trial (ALFA-0701), the addition of gemtuzumab ozogamicin (GO) to chemotherapy was assessed in 278 patients aged 50–70 years with previously untreated *de novo* AML. GO is a humanized anti-CD33 monoclonal antibody—conjugated with calicheamicin—that targets CD33-positive leukaemic cells that are commonly detected in patients with AML. The investigators chose a fractionated application of GO to produce a high cumulative dose with acceptable levels of toxicity. Patients received standard induction with or without GO and, upon complete remission or complete remission with incomplete platelet recovery (CRp), two consolidation courses with or without GO. No difference in the rates of complete remission and CRp was observed between the two groups, but patients receiving GO had significantly longer periods of relapse-free survival and overall survival (Table 1). Notably, the favourable effects of GO were strong among patients in favourable-risk or intermediate-risk cytogenetic categories, whereas patients with adverse cytogenetics did not benefit. As expected, treatment-induced neutropaenia and thrombocytopaenia were prolonged in patients receiving GO; veno-occlusive disease, which is described to be associated with GO, was reported in three of 139 patients.

Also in 2012, the results of the UK Medical Research Council (MRC) AML16 trial suggested that a single dose of GO added to induction was associated with improved outcomes in a cohort of mostly older patients (median age 67 years) with AML or myelodysplastic syndromes (MDS; Table 1).[2] In a previous MRC trial (AML15) in predominantly younger patients with AML (median age 49 years),[3] the overall survival advantage conferred by a single dose of GO during induction was—consistent with the ALFA findings—strongest among patients with favourable-risk or, by trend, intermediate-risk cytogenetics. By contrast, in a 2009 meeting abstract, no favourable effect of GO was reported in the Southwest Oncology Group S0106 trial, in which fatal events during induction were more frequent among patients receiving GO than those not receiving the drug (5.8% versus 0.8%; P=0.002).[4] Subsequently, GO was withdrawn from the US market in 2010. Approval for GO by the FDA in 2000 was based on the results of phase II studies that showed a 30% remission rate among 142 patients with AML in their first relapse.[5] However, approval was granted under the condition that post-marketing studies confirm its benefit. Although several countries granted marketing authorization for GO, the European Medicines Agency (EMA) refused approval in 2008 on the basis of an unfavourable risk-to-benefit ratio. However, based on the recent reports,[1–3] GO might enhance AML treatment, particularly as an addition to induction therapy. Although the optimal dose schedule and patient group most likely to benefit have yet to be defined, GO and other CD33 antibodies, which are currently under investigation, might change the course of treatment in patients with AML of all ages.

Among patients 60–65 years and older, few improvements in treatment of AML were made in the past few decades. One reason for the lack of advances in this age group is the paucity of therapeutic alternatives to intensive chemotherapy. One such alternative—the hypomethylating agent decitabine—was approved in 2012 by the EMA for the treatment of patients newly diagnosed with AML who are ≥65 years and who are not candidates for standard chemotherapy. Decitabine was previously approved (in 2006) by the FDA for use in patients with MDS, but the FDA refused expanded approval for treatment of AML in 2012. The decisions by the FDA and EMA were based on the study by Kantarjian *et al.*,[6] in which 485 patients ≥65 years with newly diagnosed AML received decitabine (20 mg/m^2 daily for 5 days every 4 weeks) or their treatment choice (supportive care or low-dose cytarabine). Decitabine was associated with higher rates of complete remission and CRp (17.8% versus 7.8%; P=0.001) and longer overall survival (median 7.7 months versus 5.0 months) than the alternative treatments combined. This difference in overall survival was not significant at the predefined primary analysis (HR 0.85, 95% CI 0.69–1.04; P=0.108), but it was in an unplanned updated analysis 1 year later (HR 0.82, 95% CI 0.68–0.99; P=0.037). The FDA refused authorization, in part, because the primary end point of

overall survival was not met. However, the EMA acknowledged the benefit of decitabine given the paucity of treatment options available for older patients and the relatively low toxicity.[6] Interestingly, the researchers observed that the favourable effect of decitabine on overall survival was greatest among patients who were ≥75 years or had an Eastern Cooperative Oncology Group (ECOG) performance status of 2, *de novo* disease, >30% marrow blasts or intermediate-risk[6] cytogenetics. Another 2012 report on decitabine in patients with AML >60 years suggested that patients with monosomies might also benefit from decitabine treatment.[7] Both these studies highlight the importance of defining the patients most likely to benefit from decitabine. For future clinical use, further specifying these patient groups will be important, as will designing combined regimens that include decitabine and establishing the role of this drug as a 'bridge' for patients to receive reduced-intensity conditioning and allogeneic haematopoietic stem-cell transplantation (HSCT).

Allogeneic HSCT has a pivotal role in AML treatment. A major component of treatment success is the antileukaemic effect mediated by donor T cells and natural killer cells. The function of natural killer cells is controlled by transmembrane killer cell immunoglobulin-like receptors (KIRs), the repertoire of which varies among individuals. Of the KIRs, KIR2DS1 is capable of being bound by HLA-C2, which modulates natural killer cell function. KIR2DS1 positive natural killer cells isolated from individuals expressing at least one HLA-C1 allele (*HLA-C1/C1* or *HLA-C1/C2)* are activated, while cells from individuals homozygous for HLA-C2 (*HLA-C2/C2*) are hyporesponsive.[8] Thus, matching transplant donors and recipients according to their *KIR2DS1* and *HLA-C* genotypes promises to increase the antileukaemic effects. Indeed, this premise was examined by Venstrom *et al.*[9] in their retrospective study of 1,277 patients with AML and their unrelated donors. Patients with *KIR2DS1*-positive donors had a lower cumulative incidence of relapse (CIR) than those with *KIR2DS1*-negative donors (HR 0.76, 95% CI 0.61–0.96; $P = 0.02$). Patients with *KIR2DS1*-positive donors with a *HLA-C1/C1* or *HLA-C1/C2*, rather than a *HLA-C2/C2* genotype, had lower CIR (HR 0.46, 95% CI 0.28–0.75; $P = 0.002$) and longer relapse-free survival (HR 0.68, 95% CI 0.47–0.98; $P = 0.05$).[9] Accordingly, allografts with a single *HLA-C* mismatch from *KIR2DS1*-positive donors were associated with lower CIR than those from *KIR2DS1*-negative donors (HR 0.40, 95% CI 0.20–0.78; $P = 0.007$). Overall, the study by Venstrom *et al.*[9] highlights the clinical importance of the role natural killer cells can have in preventing AML relapse after HSCT. Although the KIR-ligand interactions and the dynamics of natural killer cell activity in the recipient and effect these have on the development of graft-versus-host disease require further investigation, the available data suggest *KIR* genotyping of donors, in addition to HLA typing, would be advantageous.

Key advances

- Gemtuzumab ozogamicin and decitabine have been shown to improve outcomes in elderly patients with acute myeloid leukaemia (AML);[1,2,6] decitabine has been approved for treatment of AML in the European Union
- Relapse after allogeneic stem-cell transplantation can be reduced by selecting donors according to their killer cell immunoglobulin-like receptors *(KIR)* genotypes[9]
- Novel insights into the genetic architecture and evolution of AML have been unveiled by next-generation sequencing[10]

Treatment strategies have been greatly enhanced by the genetic characterization of AML. Next-generation sequencing technologies provide the possibility of even deeper insights into the genetic heterogeneity of AML. For example, Welch *et al.*[10] performed whole-genome sequencing on 12 patients with cytogenetically normal AML without maturation (FAB M1) and 12 patients with *PML–RARA*-positive acute promyelocytic leukaemia (FAB M3). From the analysis, 10,563 somatic variants were identified, 319 of which were located in the coding regions of 287 genes. The genes newly identified as mutated in AML and genes previously known to be affected were analysed in 84 additional patients with AML (FAB M1 or FAB M3), with the aim of identifying which genes are recurrently mutated and, therefore, most likely implicated in pathogenesis. Nine genes were recurrently mutated in both AML FAB M1 and FAB M3: *FLT3*, *NRAS*, *WT1*, *TTN*, *PKD1L2*, *CACNA1E*, *DNAH9*, *ANKRD24* and *PHF6*. Thirteen genes were mutated only in AML FAB M1 (*NPM1*, *DNMT3A*, *IDH1*, *IDH2*, *TET2*, *ASXL1*, *RUNX1*, *PTPN11*, *DIS3*, *KIT*, *SMC1A*, *SMC3* and *STAG2*). Among these mutations are several that have not yet been investigated in larger cohorts of patients with AML. The investigators demonstrated that the number of mutations in the haematopoietic stem or progenitor cells (HSPCs) from healthy individuals is comparable to that in patients with AML and increases with age. The data of Welch *et al.*[10] indicate that in many AML cases, only one or a few driver mutations occur in an HSPC that already contains hundreds of random, likely passenger, mutations.[10] Insights into the mutational landscape and the evolution of AML pinpoint molecules and pathways—as well as leukaemic clones—that can be targeted in current and future treatments.

In 2012, GO and decitabine, two drugs that have been under investigation for many years, have demonstrated a survival advantage against the respective standard treatments in AML. These drugs offer additional treatment options to patients, which are particularly needed for older individuals with AML. Moreover, a potential advance in allogeneic HSCT was revealed in matching donors and recipients according to *KIR* genotypes, in addition to HLA types, to enhance antileukaemic effects mediated by natural killer cells. Through next-generation sequencing our knowledge of the mutational patterns in AML also increased. Although the functional implications of most of the findings require further investigation, they will ultimately lead to the development of novel treatments. In 2013, we expect more data on the clinical effects of novel agents in AML, particularly those targeting kinases and epigenetic modifications. Moreover, the patient groups that most likely benefit from individual therapies will be further specified. Clinical trials, which are stratified for genetic aberrations of the leukaemic blasts, are already being conducted and promise to further our efforts to individualized treatment and improved outcomes.

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Competing interests
The authors declare no competing interests.

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TYPE 2 DIABETES MELLITUS IN 2012

Optimal management of T2DM remains elusive

Rury R. Holman

Worldwide, >366 million people with type 2 diabetes mellitus remain at excess risk of cardiovascular disease and face a lifetime of treatment escalation for this progressive disorder. Studies in 2012 have re-affirmed the safety of early insulin treatment, metformin use in renal impairment, and shown β-cell function preservation over several years.

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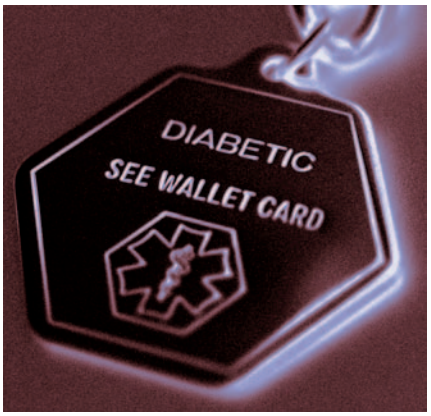
Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by progressive hyperglycaemia secondary to declining β-cell function, and usually accompanied by a reduced sensitivity to insulin. Despite the availability of an increasing number of treatment modalities for T2DM, two fundamental therapeutic issues have yet to be addressed. Firstly, people with T2DM continue to have excess cardiovascular morbidity and mortality compared with the general population. Secondly, no single therapy is yet able to maintain good glycaemic control in the long term. Questions also remain about the safety of insulin therapy with respect to vascular and cancer outcomes, and the use of metformin therapy in patients with renal impairment. This article discusses key publications from 2012 that have helped address these issues.

Individuals with T2DM remain twice as likely to develop cardiovascular disease as those without T2DM, even after adjustment for age, smoking status, BMI and systolic blood pressure.[1] Around two-thirds of patients with T2DM continue to die from coronary heart disease or stroke, despite the use of proven risk-reduction strategies that include lowering of blood pressure, levels of cholesterol and blood glucose, and smoking cessation. New and innovative approaches to tackling their residual cardiovascular risk are required. In addition, a major unmet need remains for more durable glycaemic treatments that can achieve and maintain near-normal blood glucose levels without promoting weight gain or hypoglycaemia. Such new glycaemic therapies should be simple to administer without onerous glucose monitoring requirements and, crucially, have no long-term adverse effects such as further increasing patients' cardiovascular risk.



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The ORIGIN trial, reported in 2012,[2] examined whether using insulin replacement therapy to correct the relative insulin deficiency seen in T2DM and in dysglycaemic individuals could reduce the incidence of cardiovascular events. The trial compared the use of a basal insulin (insulin glargine) to target normal fasting plasma levels of glucose (<5.3 mmol/l) with standard care, and, in a two-by-two factorial design, n-3 fatty acid supplements versus placebo. In 12,537 individuals with cardiovascular risk factors and impaired glucose tolerance, impaired fasting glucose levels or T2DM followed up for a median of 6.2 years, basal insulin supplementation had a neutral effect on cardiovascular outcomes and cancer incidence. Daily supplementation with 1 g of n-3 fatty acids did not reduce the rate of cardiovascular events.

Although ORIGIN did not show that insulin replacement therapy could reduce the incidence of cardiovascular events, the results of this trial have changed the safety landscape with respect to insulin treatment for patients with T2DM. Previous concerns that exogenous insulin therapy might increase the risks of cardiovascular disease[3] and cancer[4] have been allayed, at least for modest insulin doses used early in the disease pathway and for over 6 years. ORIGIN also demonstrated convincingly that a proactive and well-managed approach to optimizing blood glucose levels can successfully achieve and maintain near normal HbA_{1c} levels for >6 years, either with initial insulin therapy or with optimized standard care. Routine adoption of a clinical practice approach that pre-empts rises in glucose levels, rather than the current approach of serial glycaemic rescue, could do much to further offset the risk of microvascular complications and to help minimize cardiovascular events in individuals with T2DM.

In studies with ≤1 year of follow-up, intensive insulin therapy in patients with newly diagnosed T2DM has been shown to maintain β-cell function and lengthen glycaemic remission, compared with oral hypoglycaemic agents. The ORIGIN investigators did not report β-cell function, but a study by Harrison *et al.*[5] reported that β-cell function could be preserved for at least 3.5 years with intensive therapy initiated at diagnosis of T2DM, using either insulin plus metformin or triple oral therapy, after an initial 3-month insulin-based treatment period. Following the lead-in period of treatment with insulin and metformin, 58 patients with treatment-naive, newly diagnosed T2DM were allocated at random to receive insulin plus metformin or triple oral therapy with metformin, glyburide and pioglitazone. Initial mean HbA_{1c} levels of 10.8% were reduced to 6.4% with insulin and metformin and 6.6% with triple

Key advances

- Insulin treatment does not increase the risk of cardiovascular disease or cancer in patients with newly-diagnosed T2DM[2]
- A proactive approach to maintaining glycaemic control, adding therapies ahead of time rather than waiting for glycaemic targets to be breached, can maintain near-normal glucose levels for over 6 years in people with newly-diagnosed T2DM[2]
- Intensive glycaemic management from diagnosis of T2DM can help preserve remaining β-cell function for over 3 years[5]
- Metformin remains the foundation of therapy for T2DM,[6] and can be used with care even in those with renal impairment (estimated glomerular filtration rate ≥30 ml/min/1.73 m^2 and <60 ml/min/1.73 m^2)[10]

therapy, for 3.5 years, which is well below the 7.0% value recommended by many guidelines. These new findings again show that sustained near-normal glycaemia can be achieved with a proactive approach to management and the use of therapies in combination.

During the study, despite weight gains of 4 kg in the insulin plus metformin group and 10 kg in the triple oral therapy group, with an increase of the mean insulin dose in the insulin plus metformin group from 0.63 to 0.82 units/kg per day, no significant changes occurred in β-cell function between or within groups. This finding is unlike the 4% year-on-year average reduction in β-cell function seen over the first 6 years of the UK Prospective Diabetes Study in patients with newly-diagnosed T2DM receiving monotherapy with diet alone, a sulphonylurea or metformin. Hypoglycaemia rates in the study by Harrison and co-workers[5] were low and decreased over time, emphasizing that good glycaemic control can be achieved safely with intensive therapy when started at the time T2DM is diagnosed. This study supports the suggestion that early combination therapy could well be superior to the sequential approach recommended by the 2012 American Diabetes Association and European Association for the Study of Diabetes position statement.[6] The GRADE study of early treatment for T2DM, launching in 2013 and sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases, was designed to understand the relative effectiveness of different medications in combination with metformin, and to examine specifically whether introducing them sequentially or in combination from the outset would be more effective in maintaining glycaemic goals over time.[7] Regrettably, this study has been modified to only examine the sequential addition of a second agent.

Metformin remains the preferred and most cost-effective first-line pharmacological treatment for T2DM, as endorsed by the 2012 American Diabetes Association and European Association for the Study of Diabetes Position Statement.[6] It is usually continued even when other agents are added, including insulin. Metformin's use has been limited, however, by its contraindication in patients at risk of developing lactic acidosis,[8] particularly in those with renal impairment. The widespread reporting of estimated glomerular filtration rates (eGFR) by routine laboratories has increased the proportion of patients thought unsuitable for metformin therapy, given manufacturer recommendations that only those with eGFR values of ≥60 ml/min/1.73 m^2 should receive the drug. Encouragingly, the 2009 NICE guidelines are less stringent,[9] recommending that metformin doses be reviewed for patients with eGFR values <45 ml/min/1.73 m^2, but only stopping the drug for values <30 ml/min/1.73 m^2. Nevertheless, many clinicians remain unduly concerned about prescribing metformin in patients with lesser degrees of renal impairment.

In 2012, Ekström *et al.*[10] evaluated the effectiveness and safety of metformin use in 51,675 Swedish men and women aged ≥40 years to <85 years with T2DM and different levels of renal function. This observational study, with a mean follow-up of 3.9 years, was conducted in hospital outpatient clinics and primary care centres between July 2004 and December 2010. Metformin was associated with reduced risk of all-cause mortality compared with both insulin and oral hypoglycaemic agents, in line with the results of the UK Prospective Diabetes Study. Compared with any other treatment for T2DM, metformin was associated with reduced risks of acidosis and/or serious infection (adjusted HR 0.85, 95% CI 0.74–0.97) and all-cause mortality (HR 0.87, 95% CI 0.77–0.99), in patients with eGFR values of 45–60 ml/min/1.73 m^2. In particular, no increased risks of all-cause mortality, acidosis and/or serious infection or cardiovascular disease were found in those patients with eGFR values of 30–45 ml/min/1.73 m^2. Hopefully, these reassuring data will encourage the initiation or continued use of metformin in a wider range of people with T2DM.

This article has described key papers in 2012 that have attempted to address some outstanding issues in the treatment of T2DM. They have re-affirmed the safety of early insulin use, metformin treatment in renal impairment, and suggested that β-cell function can be preserved for several years. However, they have not revealed treatments that reduce the excess cardiovascular morbidity and mortality associated with T2DM, and only combination therapies have been shown to maintain good glycaemic control in the long term. Until current and future studies answer these questions, optimal management of T2DM will remain elusive.

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ADIPOSE TISSUE METABOLISM IN 2012

Adipose tissue plasticity and new therapeutic targets

Sven Enerbäck

2012 has been a rewarding year for adipocyte research. A new type of brown-like adipocyte—the beige adipocyte—and irisin, a previously unknown hormone that stimulates the formation of such cells, have been discovered. A bipotential adipocyte progenitor giving rise to both brown and white adipocytes has also been identified.

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During the course of evolution, our species has been challenged by a cold environment and by food shortages. Adipose tissue, of which there are several different types, helps humans to deal with these difficulties. While white adipose tissue (WAT) helps us to buffer fluctuations in energy availability, classic brown adipose tissue (BAT) has the unique ability to uncouple cellular respiration from the production of ATP and instead produce heat. Recent findings indicate that humans as well as rodents have not two but three types of adipocytes.[1,2] In addition to the white adipocytes that store energy in the form of triglycerides and the classic brown adipocytes that make up the interscapular thermogenic organ of many mammals, a new type of brown adipocyte has been identified —the so-called beige adipocyte (also known as brite adipocytes).[1,2] Beige adipocytes are similar to brown adipocytes and are found interspersed in WAT.

Small mammals such as rats and mice have an unfavourable relationship between their body surface area and body volume and therefore emit heat to such an extent that they frequently need an extra 'heater' to maintain normal body temperature. The interscapular thermogenic organ, which consists of interscapular brown adipose tissue (iBAT) is responsible for this heat production. As the name implies, the interscapular thermogenic organ is located between the shoulder blades just under the subcutaneous white fat and reaches down to the muscle fascia of the back muscles. The organ is made up of classic brown adipocytes that are innervated by the sympathetic nervous system (SNS). In response to cold, β_3-adrenergic receptors on iBAT cells are activated by norepinephrine released from SNS nerve endings. This initial step of a signalling pathway involving activation of protein kinase A (PKA) will lead to heat production. Ultimately, this heat production is achieved by the action of uncoupling protein 1 (UCP1), which is a protein found in the inner mitochondrial membrane of brown adipocytes that cross-circuits the proton gradient generated by the respiratory chain. Heat is produced instead of ATP as a consequence of this action.

For many years, it has been known that during prolonged exposure to cold many compartments of WAT in rodents start to look brownish.[3] A similar 'browning' effect can also be achieved by administering β_3-agonists that mimic cold exposure. In addition, adipocytes in WAT depots have been shown to undergo browning and to express UCP1 in response to treatment with thiazolidinediones.[4] The origin of the brown adipocytes responsible for the browning of WAT was unknown. Some studies suggested that white adipocytes could undergo 'brown conversion', a notion that was questioned when we learned that iBAT cells are derived from a distinct set of precursors that express the myogenic factor Myf5, whereas the brown fat cells found in WAT are derived from Myf5-negative precursors.[5] This new information opened up the possibility that the brownish cells that express UCP1 in WAT could be a distinct type of cell that is different from both white adipocytes and the classic brown adipocytes.

To explore this possibility, Wu *et al.*[1] studied undifferentiated adipose precursor cells from the stroma vascular fraction of the inguinal subcutaneous adipose tissue compartment of mice. Cell lines established from single precursor cells were allowed to differentiate into adipocytes *in vitro*. Three distinct cell-type clusters were identified on the basis of an unbiased gene expression analysis. In addition to white and brown adipocytes, a 'beige' cell type was identified that had a unique gene-expression signature and an interesting functional difference regarding expression of UCP1. Classic brown adipocytes have pronounced basal expression of UCP1 even when not stimulated by PKA-activators such as forskolin, and this basal expression is enhanced several-fold after stimulation. In beige adipocytes, the basal expression of UCP1 is similar to that of white adipocytes (which do not express UCP1), however, after stimulation, the expression of UCP1 in beige adipocytes reaches the same high level as that seen in brown adipocytes. This finding is important, as it indicates that classic brown adipocytes, unlike beige adipocytes, express a notable basal level of UCP1 as a result of an inherent function. Perhaps this feature relates to the basal sympathetic tone of the SNS (that is, the background activity by which SNS nerves release norepinephrine, even when unstimulated) and that this programme is maintained *in vitro*. The basal expression levels of UCP1 in beige adipocytes might be determined by circulating levels of β_3-agonists (that is, catecholamines released into the circulation from the adrenal medulla rather than from SNS nerve endings) and other circulating factors such as fibroblast growth factor 21,[6,7] atrial natriuretic factors[8] and irisin.[9] Consistent with this observation is the fact that

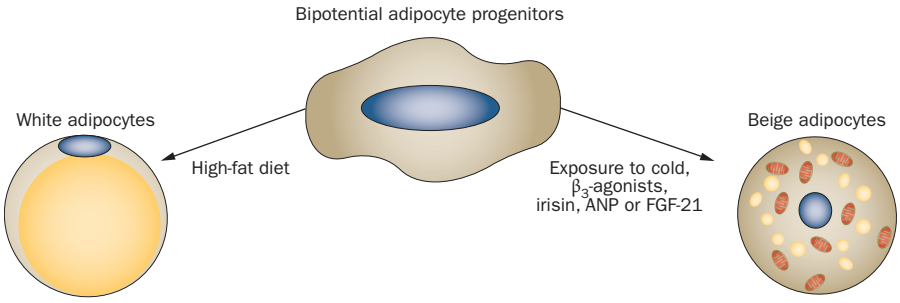

Figure 1 | A hypothetical and schematic view of how soluble factors such as irisin, FGF-21, ANP as well as cold exposure could promote differentiation of beige adipocytes whereas a high-fat diet would induce bipotential adipocyte progenitors to differentiate into white adipocytes. Abbreviations: ANP, atrial natriuretic peptide; FGF-21, fibroblast growth factor 21.

sympathetic noradrenergic nerve endings are found at a much higher density in iBAT than in WAT depots. Thus, the study by Wu *et al.*[1] provides convincing evidence for the existence of a new type of adipocyte—the beige adipocyte.

The major physiological role of WAT depots is to store energy rather than consume it through the activation of UCP1. How then is the number of UCP1-expressing beige versus white adipocytes regulated and maintained? In an interesting series of experiments, Lee and colleagues[10] address this question using genetic lineage tracing techniques. They identified a cell population in mice that expresses platelet-derived growth factor receptor α (PDGFRα), CD34 and Sca-1. These cells respond to β_3-agonists by differentiating into brown adipocytes (most likely beige adipocytes) and, in response to a high-fat diet, they become white adipocytes (Figure 1). This bipotential adipocyte progenitor thus has the capacity to act as a functional switch: if energy storage is needed after rich meals, more white adipocytes are made but if cold weather means the animal needs to produce more heat, the production of UCP1-expressing beige cells increases. Thus, the existence of a bipotential precursor offers an elegant explanation of the observed plasticity of WAT in response to cold stimulation.

When studying mice that overexpress PGC1α in muscle, Boström and co-workers[9] noted an increase in beige adipocytes positive for Ucp1 in the subcutaneous inguinal adipose tissue depot. This finding led the authors to suspect the existence of a soluble factor released from myocytes that activates browning of inguinal WAT. The factor was identified as irisin, a proteolytic fragment of the type I membrane protein FNDC5. By increasing levels of Ucp1 in subcutaneous adipose tissue, thus browning the tissue, mice with increased levels of irisin had reduced levels of obesity and insulin resistance. Irisin levels were also found to positively correlate with physical activity levels both in mice and humans.

These interesting findings open up the possibility of new exciting therapeutic approaches in which bipotential precursor cells could be stimulated to give rise to beige rather than white adipocytes. This process might be stimulated by the action of soluble factors such as irisin, which would lead to increased energy expenditure and could be used to treat diseases linked to obesity, such as insulin resistance and type 2 diabetes mellitus.

Key advances

- A third previously unrecognized type of adipocyte—the beige adipocyte—that expresses mitochondrial uncoupling protein 1 (UCP1) has been identified in white adipose tissue depots[1]
- A bipotential adipocyte progenitor has been found that gives rise to both brown and white adipocytes[10]
- A new hormone called irisin has been identified that induces the formation of beige adipocytes that express UCP1 in subcutaneous white adipose tissue[9]

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Competing interests
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NEUROENDOCRINE TUMOURS IN 2012

Insights into signalling pathways could individualize therapy

Kjell Öberg

Neuroendocrine tumours are a heterogeneous group of neoplasms with various clinical presentations, growth rates and responses to available therapies. Studies published in 2012 have provided insights into tumour-cell signalling that will increase our knowledge of tumour biology and molecular genetics, making it possible to personalize patient care.

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Neuroendocrine tumours (NETs) are heterogeneous, and the majority are metastatic at the time of diagnosis. Before 2012, medical treatment has mostly been chemotherapy-based, and has not taken into consideration varying tumour biology (such as pancreatic versus small-intestinal NETs). Dysregulation of the mTOR pathway is involved in the pathogenesis of up to 80% of human cancers, and NETs have been linked to genetic alterations that activate the mTOR pathway (Figure 1). Somatic inactivating mutations in the *MEN1* gene have been identified in 44% of sporadic pancreatic NETs, and mutations in genes of the mTOR pathway have been found in 14% of these tumours.[1] In addition, expression profiling studies in sporadic pancreatic NETs have revealed decreased expression of TSC2 and PTEN, which are known tumour suppressors that inhibit mTOR.[2] Mutations in the genes encoding menin, TSC1 or TSC2, and neurofibromin cause the inherited tumour syndromes multiple endocrine neoplasia type 1, tuberous sclerosis and neurofibromatosis, respectively.

Everolimus is a rapamycin derivative which inhibits mTOR. The RADIANT-2 and RADIANT-3 trials showed that everolimus had significant antitumour efficacy in patients with progressive pancreatic and other NETs.[3,4] In 2012, Serra and co-workers[5] found that a single nucleotide polymorphism resulting in a Gly388Arg substitution in FGFR-4 alters pancreatic NET progression and response to everolimus therapy. Everolimus inhibited mTOR phosphorylation and increased levels of phosphorylated Akt in cultured pancreatic NET cells expressing the FGFR-4 Gly388 isoform, whereas cells expressing the FGFR-4 Arg388 allele were resistant to treatment. The researchers also generated mice with pancreatic tumours expressing either the Gly388 or the Arg388 isoform of FGFR-4. Everolimus significantly delayed growth and progression of FGFR-4 Gly388 tumours, but not of FGFR-4 Arg388 tumours.

Serra *et al.*[5] genotyped 71 patients with different types of pancreatic NETs and found that 30 (42.3%) were heterozygous and six (8.5%) were homozygous for the FGFR-4 Arg388 allele. In the 43 patients who had proliferating tumours, 16 (37%) were heterozygous and 4 (9%) were homozygous for FGFR-4 Arg388. Of 12 patients who had pancreatic NETs with liver metastases, 83% had at least one copy of the FGFR-4 Arg388 allele. Patients with the FGFR-4 Arg388 allele were more likely to develop liver metastases (OR 6.3, 95% CI 1.3–31.5) than patients homozygous for the FGF-R Gly388 allele. Regression analysis confirmed the FGFR-4 Arg388 allele as a predictive marker of liver metastases. Additionally, the FGFR-4 Arg388 allele was present in 83% of patients with stage IV pancreatic NETs.

Serra and colleagues[5] also genotyped 17 patients with stage IV pancreatic NETs who had received everolimus in a previous trial. They found that 11 (65%) patients were homozygous for the FGFR-4 Gly388 allele, whereas 6 (35%) patients carried an FGFR-4 Arg388 allele. Following everolimus treatment, patients homozygous for the FGFR-4 Gly388 allele had larger mean reductions in tumour size (25% versus 9%), greater than threefold longer progression-free survival (16.6 months versus 4.8 months) and more than fourfold longer overall survival (40 months versus 9.3 months) than those with the FGFR-4 Arg388 allele. This study is of particular interest because it shows for the first time that a specific gene polymorphism significantly modifies the behaviour of pancreatic NETs and their response to a specific treatment.

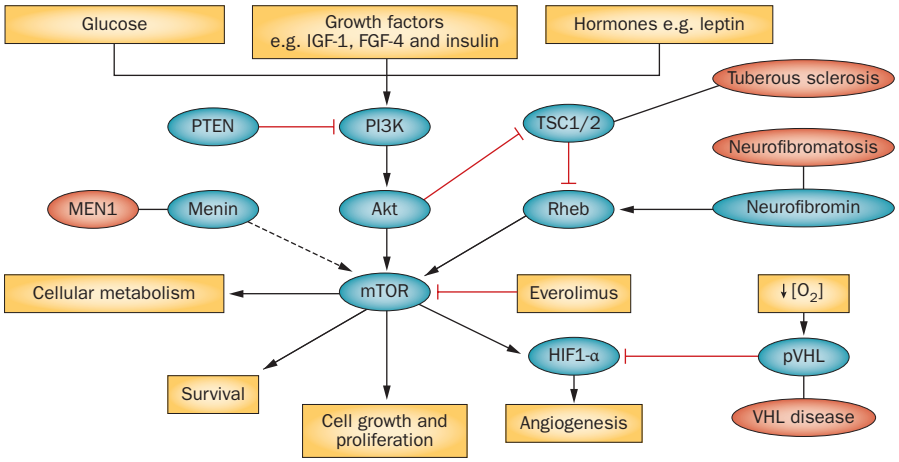

Figure 1 | Many neuroendocrine tumours harbour mutations in genes encoding members of the mTOR pathway. Some inherited tumour syndromes (red) are caused by mutations in these genes. Abbreviations: MEN1, multiple endocrine neoplasia type 1; VHL, von Hippel–Lindau.

ATRX and *DAXX* are tumour suppressor genes that encode the nuclear proteins ATRX and DAXX, which are thought to be involved in chromatin remodelling at telomeric and pericentromeric regions of chromosomes. Mutations in these genes are tightly associated with loss of nuclear expression of their respective proteins, and correlate with the alternative lengthening of telomeres phenotype. In a study by de Wilde and co-workers,[6] samples from 28 patients with MEN1 were tested for mutations in either the *ATRX* or *DAXX* genes. Patients with MEN1 are predisposed to the development of pancreatic NETs and the pancreata of these patients usually harbour multiple neuroendocrine microadenomas <0.5 cm, which are thought to represent precursors to pancreatic NETs. In this study, samples from a total of 109 lesions were analysed (1–11 lesions per patient). Of these, 47 were samples from microadenomas, 50 were from pancreatic NETs and 12 were of pancreatic NET lymph node metastases. ATRX and DAXX expression was defective, and the alternative lengthening of telomerase phenotype was present in 3% of neuroendocrine lesions of pancreatic NETs but not in any of the 47 microadenomas examined. Three of 50 (6%) pancreatic NETs ≥0.5 cm had defective ATRX or DAXX expression. Two of the three tumours were from a patient who had concurrent lymph node metastases with the same changes as the primary tumour. Loss of ATRX or Daxx nuclear expression and the alternative lengthening of telomeres phenotype occurred only in large pancreatic NETs (>3 cm) of patients with MEN1, indicating that these changes are late events in pancreatic NET tumourigenesis.

In a study by Speisky and co-workers,[7] expression of genes related to angiogenesis (in the HIF–VHL pathway), epithelial–mesenchymal transition or metastasis were analysed at the mRNA and protein level in pancreatic NETs. In the study, 18 patients had the inherited tumour syndrome von Hippel–Lindau (VHL) disease (Figure 1) and 16 patients did not. Expression levels of some proteins were compared with those in microadenomas, which represent the early NET precursor. In tumours from patients with VHL disease, 19 of the 52 genes analysed (36%) were significantly upregulated in the tumours and the same 19 genes were upregulated in the tumours of the group of patients without VHL. The upregulated genes were directly related to HIF signalling, angiogenesis, epithelial–mesenchymal transition, metastasis, growth factor signalling and the cell cycle. Three of the 52 genes were significantly downregulated in the tumours of the patients with VHL and two of these were also downregulated in tumours of patients without VHL. The downregulated genes were involved in epithelial–mesenchymal transition and angiogenesis signalling pathways. Of note, strong mRNA expression of *VEGFA* and its encoded protein's receptors (*VEGFR1* and *VEGFR2*) were noticed in tumours of patients with VHL disease. On the basis of these results, VEGF signalling is a prime therapeutic target.

A large, randomized, placebo-controlled study has demonstrated the efficacy of sunitinib, a potent tyrosine-kinase inhibitor that targets the VEGF pathway, in

Key advances

- A single nucleotide polymorphism in *FGFR4* can alter pancreatic neuroendocrine tumour (NET) progression and response to treatment with the mTOR inhibitor everolimus[5]
- Altered expression of the tumour suppressors *ATRX* and *DAXX* is a late event in pancreatic NETs of patients with multiple endocrine neoplasia type 1[6]
- The VEGF–HIF pathway is central in progression of both sporadic and inherited NETs[7]
- NOTCH1 acts as a tumour suppressor and is important for preventing rectal carcinoid tumourigenesis[9]

patients with pancreatic NETs.[8] The results of this study support the molecular basis of using tyrosine-kinase inhibitors in the management of patients with pancreatic NETs related to VHL disease and in sporadic NETs.

Wang and co-workers[9] performed immunohistochemistry analyses and gene expression profiling on biopsied tissue from well-differentiated NETs of the pancreas ($n = 74$), small intestine ($n = 31$) and rectum ($n = 15$). *NOTCH1*, which encodes Notch 1, was expressed in all rectal NETs and 34% of pancreatic NETs, but all small intestinal NETs lacked *NOTCH1* expression. The downstream Notch 1 effector, transcription factor HES-1, was expressed in 65% of rectal NETs, 10% of pancreatic NETS and no small intestinal NETs, supporting the functional activity of Notch 1 in rectal NETs. The high level of *NOTCH1* expression in rectal NETs, together with the observation that 5-year survival of patients with rectal NETs is better than that of patients with other NETs, suggests that Notch 1 has a role as a tumour suppressor in these tumours. This study also shows that microarray analyses can be used on formalin-fixed and paraffin-embedded tissue. Such analyses will enable study of archival NET specimens.

Notch 1 inhibitors are unlikely to have beneficial effects in patients with small intestinal NETs owing to the lack of *NOTCH1* expression in these NETs. Instead, compounds that activate Notch 1 might be effective in ileal NETs. An ongoing trial is examining the role of resveratrol (a compound that activates Notch 1) in NETs of the gastrointestinal tract.[10]

The data generated in 2012 regarding signalling pathways in NETs provide important background for understanding tumour biology in various NET subtypes, opening the possibility of tailoring treatment to different subtypes of tumours and personalizing medicine. For the first time, we have an understanding of the mechanism by which mTOR and tyrosine-kinase inhibitors work in patients with NETs. However, many of these preclinical and clinical observations are yet to be confirmed in randomized clinical trials.

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Competing interests
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BONE METABOLISM IN 2012

Novel osteoporosis targets

Claes Ohlsson

Researchers are trying to develop more efficient and safer antifracture treatments. Besides the ongoing promising clinical trials involving antibodies to the Wnt antagonist sclerostin or inhibition of the osteoclast enzyme cathepsin K, the year 2012 has seen several novel osteoporosis targets identified by using different methodological approaches.

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Osteoporosis-related fractures constitute a major health concern and result in a huge economic burden on health-care systems. The major determinants of fracture risk include BMD, bone quality parameters and nonbone parameters (muscle mass and function, as well as balance, which influence risk of falls; Figure 1). In an important article published by Estrada and co-workers from the GEFOS (GEnetic Factors for OSteoporosis) consortium in 2012, a hypothesis-free approach was used to identify several new loci associated with BMD.[1]

The authors performed the largest genome-wide association study (GWAS) meta-analysis on BMD to date, including 17 GWASs and 32,961 individuals. The most significant genetic variants identified in this analysis were selected for replication in 50,933 participants. The researchers identified 24 known and 32 novel loci that were reproducibly associated with BMD. Several of these loci cluster within signalling pathways known to be associated with bone regulation, such as RANK–RANKL–osteoprotegerin and Wnt, or pathways of mesenchymal stem cell differentiation or endochondral ossification. However, quite a few loci were located to genes not known to have a role in bone biology. Extensive further functional studies are required to

characterize these potential osteoporosis targets. The functional translation of findings from GWAS is often challenging, but for the *WNT16* locus, which was convincingly associated with BMD and forearm fracture risk, functional studies published in 2012 using *Wnt16*-deficient mice confirmed that WNT16 is crucial for bone mass and strength.[1,2]

Of the identified BMD-associated loci, 14 were also associated with fracture risk,[1] and the authors developed a genetic risk score based on these loci. However, the clinical usefulness of this risk score for fracture prediction was limited.[1] This finding is disappointing but not unexpected, given the small fraction of the genetic risk for fracture that has been identified thus far. The most important lesson from this GEFOS publication is that it sheds light on the genetic architecture and pathophysiological mechanisms underlying BMD variation.

Twin studies have demonstrated that heritability of osteoporosis-related fractures is age-dependent, being less pronounced in elderly individuals.[3] Importantly, the heritable component of fracture risk is largely independent of BMD.[3,4] Consequently, bone researchers have proposed that numerous genes exert their effect on fracture susceptibility independently of BMD (Figure 1). This point is relevant because all antifracture treatments, currently available or being evaluated in clinical trials, target bone mass. Thus, novel treatments that target BMD-independent fracture mechanisms might be useful alone or in combination with existing treatments, especially for patients at high risk of fracture. Ongoing large-scale GWAS meta-analyses focusing on fracture risk might identify the first BMD-independent fracture targets (Figure 1).

A completely different methodological approach for the identification of new osteoporosis targets was used by Hayashi and co-workers in 2012.[5] They hypothesized that cultured mouse osteoblasts secrete proteins in addition to osteoprotegerin (a potent osteoclast inhibitor) that regulate bone metabolism. The authors fractioned the conditioned medium from cultured osteoblasts derived from osteoprotegerin-deficient mice and identified semaphorin-3A to be an osteoblast-derived protein that inhibits osteoclast formation. Importantly, this protein was shown not only to suppress osteoclastic bone resorption but also to increase osteoblastic bone formation. Semaphorin-3A-deficient mice were osteopenic, and intravenous semaphorin-3A administration prevented ovariectomy-induced bone loss.

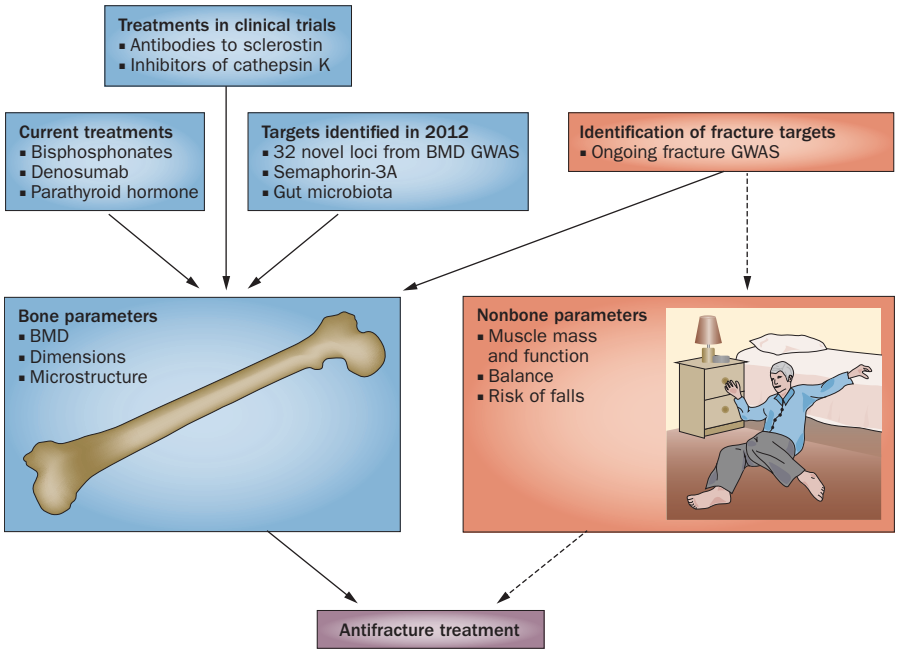

Figure 1 | Current and future antifracture treatments. The figure summarizes research themes from 2012 related to bone and nonbone parameters that shed light on targets for osteoporosis antifracture treatments. Abbreviation: GWAS, genome-wide association studies.

Semaphorin-3A reduced bone resorption and increased bone formation via different signalling pathways. The inhibitory effect on bone resorption was mediated via binding of semaphorin-3A to neuropilin-1, which prevented RANKL-induced osteoclast differentiation by inhibiting the immunoreceptor tyrosine-based activation motif and RhoA signalling pathways. By contrast, the stimulatory effect of semaphorin-3A on bone formation was mediated by the promotion of the canonical Wnt/β-catenin signalling pathway. Current treatments for osteoporosis fail to uncouple bone resorption and formation. Antiresorptive treatments inhibit bone resorption and at the same time, albeit to a smaller extent, also inhibit bone formation, whereas anabolic treatments increase bone formation and at the same time, albeit to a smaller extent, also increase bone resorption.[5,6] Thus, the mechanism of action of semaphorin-3A is highly unusual because it uncouples the two components of bone remodelling, which could be exploited for therapeutic advantage and might lead to a new class of dual-action therapeutic agents for osteoporosis.[5,6]

In another study published in 2012, using a different methodological approach, Sjögren and co-workers hypothesized that the bacteria in the gut might influence bone metabolism.[7] The human gut is populated by trillions of bacteria, known as the gut microbiota, which collectively contain 150-fold more genes than our human genome. The gut microbiota is acquired at birth and, although a distinct entity, has co-evolved with the human genome and can be considered a multicellular organ that communicates with and affects its host in numerous ways.[8] The composition of the gut microbiota is modulated by a number of environmental factors throughout life, such as diet and antibiotic treatment. Several diseases have been associated with dysregulation of the gut microbiota, including Crohn's disease, colon cancer, asthma, diabetes mellitus and obesity.

Key advances

- A large-scale, genome-wide association study meta-analysis on BMD has shed light on the genetic architecture and pathophysiological mechanisms underlying BMD variation[1]
- Semaphorin-3A is an osteoblast-derived secreted protein that increases bone mass, both via reduced osteoclast differentiation and increased bone formation[5]
- The gut microbiota regulates bone mass in mice and might be a novel therapeutic target for osteoporosis treatment and prevention[7]
- 2-year treatment with zoledronic acid reduces the risk of vertebral fractures in men with osteoporosis[10]

Sjögren and co-workers used a germ-free mouse model to test their hypothesis, and demonstrated that these mice exhibit increased bone mass compared with control mice and that the colonization of germ-free mice with a normal gut microbiota rapidly normalizes bone mass.[7] Osteoclast formation was reduced in the germ-free mice and the effects on osteoclast-mediated bone resorption were associated with reduced expression of the osteolytic cytokine TNF in bone and decreased frequency of $CD4^+$ T cells in bone marrow. The authors proposed that the inhibitory effect of the gut microbiota on bone mass is mediated via effects on the immune status in bone, which in turn regulates osteoclast-mediated bone resorption. A role of gut microbiota in the regulation of bone mass is supported by another study from 2012 that demonstrated that antibiotic treatment in early life, which alters the composition of gut microbiota, increases bone mass in young mice.[9] These studies warrant further investigations to evaluate the gut microbiota as a potential novel therapeutic target for osteoporosis.

The newly identified promising osteoporosis targets described above will hopefully lead to the development of more potent and safer antifracture treatments. Reporting in 2012 on an already approved treatment strategy for osteoporosis, with documented antifracture effect in women, Boonen and co-workers showed that the bisphosphonate zoledronic acid also reduces fracture risk in men.[10] This finding is important as osteoporosis causes morbidity and mortality in men as well as women. Previous treatment studies involving men with osteoporosis have focused on the surrogate outcomes of BMD and bone turnover markers, but data from double-blind, randomized studies assessing the antifracture efficacy as the primary end point have been lacking. The key publication by Boonen and co-workers demonstrated that 2-year treatment with zoledronic acid significantly reduced the risk of vertebral fractures among men with osteoporosis.[10] Although this finding does not imply that all data on drugs for osteoporosis in women can be extrapolated to men, they provide the confidence to proceed with further trials.[10]

In summary, notable progress in the identification of possible novel osteoporosis targets occurred in 2012. Of particular importance is the finding that the osteoblast-derived factor semaphorin-3A both reduces bone resorption and increases bone formation in rodents. If also true in humans, it could hold the key to the development of a new class of dual-action therapeutic agents for osteoporosis.[5] Recently initiated fracture-focused studies, such as ongoing GWAS meta-analyses on fracture risk, could lead to the identification of novel targets for the development of the first BMD-independent antifracture treatment (Figure 1).

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Competing interests
The author declares no competing interests.

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THYROID IN 2012

Advances in thyroid development, hormone action and neoplasia

V. Krishna K. Chatterjee

In 2012, we learned that functional thyroid tissue can be generated *in vitro*, and that thyroid hormones stimulate autophagy. Patients with defects in TRα have been identified, and di-iodothyropropionic acid has been shown to ameliorate MCT8 deficiency. Finally, we found that gene expression profiling can identify benign thyroid nodules.

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Hypothyroidism affects ~3% of the population, and patients require lifelong thyroid hormone replacement. As the thyroid gland produces a combination of T_4 and T_3, conventional therapy with levothyroxine (T_4) alone is not a complete physiological replacement and fails to fully restore well-being in some patients. In this context, the generation of functional thyroid tissue by reprogramming embryonic stem cells, as described by Antonica *et al.*[1] in 2012, is a substantial advance. Ectopic expression of *Nkx2-1* and *Pax8* in murine embryonic stem cells promoted their differentiation into thyroid follicular cells that expressed many genes mediating hormone biosynthesis, including *Slc5a5* (encoding the Na^+/I^- symporter, Nis), *Tg*, *Tpo* (encoding the proteins of the same name) and *Tshr* (encoding TSH-R; Figure 1). Remarkably, exposure of the thyroid follicular cells to TSH led to them forming 3D structures resembling thyroid follicles that can take up and organify iodide.

When the cultured thyroid tissue was implanted into hypothyroid mice, thyroid hormone levels were restored. Antonica *et al.*[1] provided extraordinary proof-of-concept that, as has been shown for the anterior pituitary,[2] ectopic expression of transcription factors, with or without a specific growth factor milieu, is capable of directing both stem-cell differentiation and high-order organogenesis that virtually recapitulates native endocrine tissue. If the technology can be applied to induced pluripotent stem cells derived from patients with idiopathic thyroid dysgenesis or dyshormonogenesis, valuable pathogenetic insights could be gained. In addition, hormone replacement therapy could conceivably be useful in patients with thyroid failure involving autologous implantation of reprogrammed thyroid cells.

Key advances

- Expression of ectopic transcription factors in embryonic stem cells can reprogram them into thyroid follicles that form functional thyroid tissue *in vivo*[1]
- Thyroid hormone signalling induces autophagy, which enhances hepatic lipid oxidation[4]
- Mutations in the gene encoding TRα in humans result in tissue-specific features of hypothyroidism, but near-normal thyroid function tests[6]
- Therapeutic use of the thyroid hormone analogue di-iodothyropropionic acid ameliorates hypermetabolism and weight loss in patients with defects in the thyroid hormone transporter MCT8[8]
- Gene expression profiling of indeterminate thyroid nodules aids identification of benign lesions and could prevent unnecessary thyroid surgery[9]

Classically, autophagy has been characterized as a catabolic pathway which involves lysosomal degradation of proteins and organelles. However, other cellular components, such as endocytosed triglyceride-rich lipid droplets, are now recognized targets of this process, with lipophagy regulating cellular nutrient availability.[3] Extending this observation, in 2012 Sinha and colleagues[4] established a link between lipophagy and thyroid-hormone action in the liver. Sinha *et al.*[4] showed that T_3 promotes lipophagosome formation and fatty-acid oxidation in the liver. Transgenic mice with a mutant TRβ that causes resistance to the actions of thyroid hormone have defective hepatic lipophagy. Additionally, short-interfering-RNA-mediated knockdown of *Atg5*, which encodes a key protein in this pathway, blocks lipophagy. These insights are relevant to understanding hepatic lipid homeostasis (including the known association between hypothyroidism and hepatic steatosis), and might also indicate a broad link between thyroid hormone signalling and autophagy in other physiological contexts.

Distinct thyroid hormone receptor subtypes (TRα1, TRβ1 and TRβ2) mediate thyroid hormone action in different tissues. Negative feedback regulation of thyroid hormone signalling within the hypothalamic–pituitary–thyroid axis is mediated predominantly by TRβ2. Resistance to thyroid hormone (RTH) is a disorder associated with heterozygous, dominant-negative mutations in the gene encoding TRβ and is, therefore, readily diagnosed owing to patients having elevated circulating thyroid hormone levels but non-suppressed TSH levels. Transgenic mouse models have been generated that harbour TRα1 defects that are analogous to the TRβ mutations found in patients with RTH.[5] In the first report of a human disorder resulting from defects in TRα1, Bochukova *et al.*[6] have now described a child with a dominant-negative mutation in the gene encoding TRα1.

The patient described has lower-segmental growth retardation; severe constipation due to slowed intestinal transit; and skeletal abnormalities, including delayed cranial-suture fusion and tooth eruption, and femoral epiphyseal dysgenesis. Remarkably, although these features are characteristic of severe hypothyroidism, the patient's circulating thyroid hormone and TSH levels were near normal. However, in contrast with patients who have classical, TRβ-mediated RTH, the patient experienced prompt TSH suppression but reduced heart rate elevation in response to levothyroxine therapy. These responses are consistent with retention of sensitivity to thyroid hormone within the pituitary–thyroid axis, but hormone resistance in the myocardium, which expresses TRα. Subsequently, an analogous TRα1 defect has been identified in a father and child with short stature, constipation and similar thyroid biochemistry to the patient identified by Bochukova *et al.*[7]

This human disorder might have eluded discovery owing to affected patients having near-normal thyroid function test results. However, an abnormal T_4:T_3 ratio and low reverse T_3 levels provide a biochemical signature that might enable future identification of patients with this disorder. Additionally, identification of these patients substantiates the existence of tissue-selective hypothyroidism without a dysregulated pituitary–thyroid axis in humans. The preservation of male fertility in an affected individual and the transmission of this disorder[7] suggest that individuals with TRα gene mutations could be common in the population. The phenotypes of these patients might be variable and be milder than features in patients who have been identified to date, depending on the type and location of the receptor defect, as has been seen in mice with different TRα1 mutations.[5] Perhaps complete understanding of the phenotypic spectrum of this disorder awaits identification of unsuspected cases from widespread, whole-genome, population sequencing studies.

Defects in the gene encoding the thyroid hormone transporter MCT8 cause

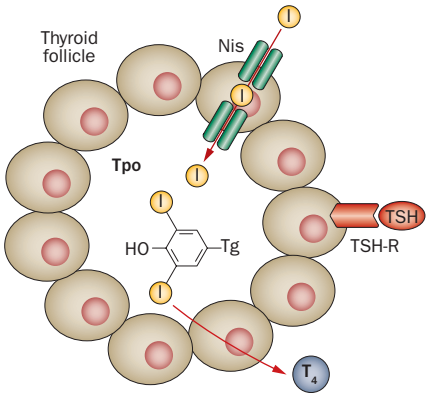

Figure 1 | Synthesis of thyroid hormone in thyroid follicles. Iodine enters follicles via Nis (Na^+/I^- symporter), is oxidized by Tpo and is organified through its incorporation into Tg. TSH binds to TSH-R, stimulating production and release of thyroid hormones. Proteolysis liberates thyroid hormones, enabling release.

Allan–Herndon–Dudley syndrome, which is characterized by marked tissue-selective (central nervous system) hypothyroidism. Patients with this disorder have a disproportionately mild pattern of thyroid dysfunction (low T_4, high T_3 and low reverse T_3 levels) that is similar to that of patients with mutations in the gene encoding TRα. However, because cellular thyroid hormone transport depends on various transporters in different tissues, the phenotype of patients in whom only MCT8 is defective is complex, with thyroid hormone deficiency and thyroid hormone excess states coexisting in different tissues.

In 2012 Verge and colleagues[8] reported the results of a study in which 3,5-di-iodothyropropionic acid (DITPA), a thyroid hormone analogue, was used to manage patients with Allan–Herndon–Dudley syndrome. Four children with confirmed mutations in the gene encoding MCT8 were treated with DITPA for 26–40 months. Therapy reduced serum T_3 levels, heart rate and levels of sex hormone binding globulin, a hepatic marker of thyroid hormone action. Therapy also induced weight gain or reduced weight loss such that supplementary enteral feeding was not required. These results provide proof-of-principle that hormone analogue treatment is efficacious in patients with MCT8 deficiency. Unfortunately, because the central-nervous-system-based phenotype of this disorder probably reflects neuronal hormone deficiency during fetal development, it was largely irreversible. However, these results raise the intriguing possibility that starting treatment early in development

(for example *in utero*) could circumvent the transporter defect and ameliorate or prevent the devastating neurological sequelae of this disorder.

With the use of ultrasonography, thyroid nodules are identified with increasing frequency (in 25–50% of adults). However, the vast majority (~90%) of these are benign—an important management problem in clinical practice. Following diagnosis of malignancy after analysis of samples obtained using fine-needle aspiration biopsy (FNAB), surgery is recommended. Unfortunately, in up to 30% of cases, cytopathology of samples obtained with FNAB is indeterminate. Therefore, many patients are subjected to thyroidectomy which is subsequently proven to have been unnecessary following a benign postoperative histological diagnosis. In 2012, Alexander *et al.*[9] showed that gene-expression profiling of FNAB samples that had been categorized as indeterminate enabled reclassification of benign samples with high (~95%) negative predictive value. Given the existence of a small false-negative rate, the most prudent use of this diagnostic test might be to discriminate between suspicious nodules that require surgery and the majority of nodules that are probably benign and can be safely monitored for change in size or character. Long-term follow-up studies are needed to validate this gene-expression classifier, but application of the test in routine clinical practice in one US centre has already reduced surgical intervention by 10-fold.[10] In the future, testing samples obtained using FNAB for molecular markers of malignancy, such as mutations in *BRAF*, *RET/PTC*, *PAX8* or *PPARγ*, is likely to improve the diagnostic efficacy of the procedure.

In 2012, remarkable advances have been made in the thyroid field. Although development of the thyroid gland *in vivo* is complex, thyroid organogenesis can be achieved fairly simply *in vitro* by reprogramming stem cells. Coexistence of tissue hypothyroidism and hyperthyroidism in individuals with defects in genes encoding thyroid hormone receptors or thyroid hormone transporters emphasizes the diversity of proteins which mediate these processes. Additionally, molecular profiling has improved the classification of thyroid neoplasms, thus preventing unnecessary surgical intervention.

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Competing interests
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CARDIOVASCULAR ENDOCRINOLOGY IN 2012

PCSK9—an exciting target for reducing LDL-cholesterol levels

D. John Betteridge

Systemic administration of anti-PCSK9 antibodies induces dramatic reductions in LDL-cholesterol levels, and the effect of this therapy on LDL-receptor activity seems to be additive to that of statin therapy. Inhibition of PCSK9 is potentially very important to the clinician, and should enable more patients to achieve their LDL-cholesterol-level goal.

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Introduction of the statin class of drugs into clinical practice in the late 1980s transformed the management of cardiovascular diseases (CVDs). These drugs inhibit the rate-determining enzyme in cholesterol synthesis HMG-CoA reductase. As a result of this inhibition, hepatic expression of the LDL receptor (LDLR) is increased and plasma concentrations of LDL cholesterol are reduced. The statin class of drugs proved to be well tolerated and highly effective in lowering LDL-cholesterol levels in patients with CVD. This success enabled robust randomized placebo-controlled trials to be performed to test the capacity of these drugs for both primary and secondary prevention of coronary events.[1] A wealth of efficacy and safety data now exist from randomized placebo-controlled trials to guide therapy across a wide spectrum of disease areas that are associated with increased CVD risk.[1] Furthermore, it has become clear that intensive LDL-cholesterol-level lowering, achieved either through the use of a high-dose statin or a statin with high efficacy, results in a significantly greater reduction in CVD risk than does treatment with a low-dose statin or a less effective statin.[2]

Statins are recommended as the first-line therapy for reducing levels of LDL and other apolipoprotein B-100-containing lipoproteins. Over the past decade, goals of therapy have become more stringent.

Key advances

- Antibodies that inhibit proprotein convertase subtilisin/kexin type 9 (PCSK9) reduce LDL-cholesterol levels[6,7]
- In terms of reducing LDL-cholesterol levels, treatment with a PCSK9-targeting antibody is additive to statin therapy in patients with familial or primary hypercholesterolaemia[8,9]
- In patients who are intolerant to statin therapy, an antibody targeting PCSK9 reduces LDL-cholesterol levels[10]

For those at very high risk of developing CVD, an LDL-cholesterol-level goal of <1.8 mmol/l is recommended. In patients with type 2 diabetes mellitus or other conditions associated with mixed lipidaemia, a target non-HDL-cholesterol level of <2.6 mmol/l has been advocated as the secondary target, acknowledging that potentially atherogenic cholesterol is present on lipoproteins other than LDLs, such as intermediate-density lipoproteins and remnant particles.[3]

Despite the widespread clinical use of statins, achieving LDL-cholesterol-level goals in clinical practice remains a challenge. For example, these goals might be difficult to achieve in individuals who have very high baseline LDL-cholesterol levels because of severe primary dyslipidaemias (such as familial hypercholesterolaemia, familial combined hyperlipidaemia or type III dysbetalipoproteinaemia); in those with very high CVD risk who require intensive therapy goals; or in patients for whom statin dosage is limited owing to potentially serious drug interactions, comorbid conditions or intolerance of high drug doses. Additionally, some individuals cannot or will not take statins for a variety of reasons, and in many lipid clinics this problem represents a major referral pattern. The percentage of individuals who are intolerant to statin treatment is difficult to define, but from my experience as a clinician this percentage is likely to be ~10%. Therefore, despite the availability of statins, which are one of the most important innovations in any branch of medicine, a substantial number of patients remain who are unable to reach treatment goals through the use of these drugs. An exciting advance in the past decade is that detailed study of genetic mutations affecting cholesterol metabolism has led to the identification of new therapeutic targets. The serine protease proprotein convertase subtilisin/kexin type 9 (PCSK9; Figure 1) is just one such example of a promising target.

The activity of hepatic LDLRs is the major determinant of plasma LDL concentration, and a study of families with a severe familial hypercholesterolaemia phenotype identified a previously unknown cellular process that is important for LDLR activity.[4] PCSK9, a serine protease synthesized primarily in the liver, reduces the number of LDLRs in hepatocytes. Circulating PCSK9 binds to the LDLR on the cell surface, is internalized with it and promotes its lysosomal degradation. Thus, as a result of PCSK9 activity, LDLR numbers are reduced and plasma LDL levels increase. Gain-of-function mutations in *PCSK9* produce a severe familial hypercholesterolaemia phenotype, whereas loss-of-function mutations in this gene have been linked to reduced plasma LDL concentrations from birth and decreased CVD risk. Of interest, PCSK9 levels increase during statin therapy.[5]

Identification of a role for PCSK9 in the regulation of LDLR activity has provided a new and very exciting target for therapeutic intervention. A reflection of this interest is that results of phase I and, subsequently, phase II studies of PCSK9 inhibitors have been published this year in major journals. PCSK9 has been targeted with specific antibodies which bind to PCSK9 and block its interaction with the LDLR. The positive results of several phase I single-dose studies in which two different specific human monoclonal antibody preparations, REGN727/SAR236553 (REGN727)[6] and AMG145,[7] were used in healthy individuals point to the huge potential of this approach, with reductions of ~60% in LDL-cholesterol levels reported in antibody-treated compared with levels in placebo-treated individuals. The degree and duration of the effect of anti-PCSK9 antibody treatment were dose-dependent, with higher dosages leading to more long-term and greater reductions in LDL-cholesterol levels than low doses.

In two 12-week randomized double-blind placebo-controlled phase II trials, the efficacy and safety of administering REGN727 in increasing doses, and at different dosing intervals, were investigated. In one study, 77 patients heterozygous for familial hypercholesterolaemia who were receiving statin therapy were included,[8] and 183 patients with primary hypercholesterolaemia who were receiving statin therapy were recruited to the other study.[9] In the patients with familial hypercholesterolaemia, 150 mg of REGN727 administered every two weeks as an add-on to statin therapy reduced LDL-cholesterol levels by 67.9% and levels of apolipoprotein B-100, the major protein carrying atherogenic cholesterol, by 50.19%. Concurrently, levels of apolipoprotein A-I, the major protein of HDL, and HDL cholesterol were increased by 8.82% and 12.34%, respectively.[8] The mechanism underlying this effect remains to be determined, but these results could indicate a reduction in the rate of cholesterol transfer from HDL to LDL via cholesterol ester transfer protein. In the study by McKenney *et al.*,[9] results were similar although levels of HDL cholesterol and apolipoprotein A-I were reduced following treatment with REGN727. Interpreting the differences between the results of these studies is difficult and probably inappropriate at this stage owing to the small number of patients involved and the short duration of both studies. The effect of using this monoclonal antibody as an add-on to statin therapy was additive, not synergistic, in both studies. As PCSK9 levels are increased in patients undergoing statin therapy, it might have been expected that a more-than-additive effect would have been seen when PCSK9 was inhibited, but such a response was not observed.

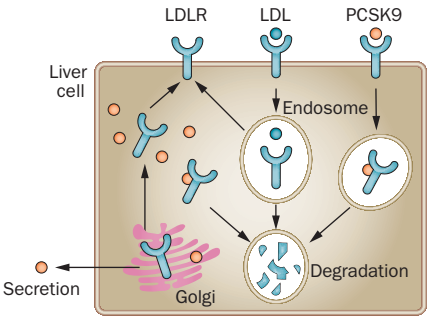

Figure 1 | PCSK9 binds the LDL receptor (LDLR), targeting it for degradation. Inhibiting PCSK9 lowers LDL-cholesterol levels by reducing LDLR degradation.

Clearly, this new therapeutic approach will be of great benefit in patients who are intolerant to statin treatment, and this population was studied in the Goal Achievement after Utilizing an anti-PCSK9 antibody in Statin Intolerant Subjects (GAUSS) trial.[10] Patients who were unable to tolerate statin treatment, owing to experiencing myalgia or myopathy, were randomized to receiving either the monoclonal anti-PCSK9 antibody AMG145 or a placebo at 4-week intervals for 12 weeks, either alone or in combination with 10 mg ezetimibe per day. The effects of AMG145 were dose-dependent, with the greatest reduction (51%) seen in patients receiving 420 mg, the highest dose used in the study. The effect of AMG145 treatment was additive to that of ezetimibe therapy, and treatment of patients with 420 mg of AMG145 and 10 mg of ezetimibe per day reduced LDL-cholesterol levels by 63%. This reduction in the levels of LDL cholesterol in patients who are statin-intolerant is dramatic and should enable this not-insignificant group of patients to be managed effectively.

The early efficacy data regarding these monoclonal antibodies that have been

published in 2012 are very impressive, but the studies performed so far are of short duration and involve small numbers of patients. Long term efficacy and safety data are required before these drugs can potentially be licensed and introduced into clinical practice. The usual safety data that we are used to reviewing in the lipid-lowering therapy field look encouraging, but what of the potential safety issues associated with monoclonal antibodies? In the study of patients with primary hypercholesterolaemia, one 57-year-old male who received an intial dose of 300 mg of REGN727 complained of diarrhoea and then a rash, which was diagnosed after biopsy as leucocytoclastic vasculitis.[9] This generally benign condition has been reported in patients undergoing therapy with other antibodies.

Another consideration is whether mild injection-site reactions will affect compliance in some patients. Indeed, will subcutaneous injection, albeit at intervals of 2–4 weeks, be readily accepted by patients? Certainly GLP-1 agonists that are given by subcutaneous injection to individuals with type 2 diabetes mellitus seem to be generally accepted by these patients. Another enormous factor that might determine how often these drugs are likely to be used will be the cost:benefit ratio of this new approach, as assessed by health-care providers. I would imagine that most providers would accept their use in patients with severe dyslipidaemias who have not met LDL-cholesterol-level goals when receiving maximum statin therapy and ezetimibe. However, will providers require patients to meet a robust definition of statin intolerance before allowing therapy with monoclonal PCSK9 antibodies? We should follow progress in this new therapeutic area with great interest.

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Competing interests
The author has declared associations with the following companies: Aegerion, Amgen, Astra Zeneca, Janssen, Kowa, MSD, Pfizer and Takeda. See the article online for full details of the relationships.

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IBD IN 2012

Pathogenesis and management of IBD—thinking outside the box

Séverine Vermeire and Paul Rutgeerts

In 2012, important advances were made in understanding the pathogenesis of IBD—the Immunochip project increased the number of known IBD loci to 163, and underscored the common susceptibility to infectious diseases. With regard to management of IBD, novel non-anti-TNF agents have shown efficacy in phase II and III trials.

Vermeire, S. & Rutgeerts, P. *Nat. Rev. Gastroenterol. Hepatol.* **10**, 67–69 (2013); published online 8 January 2013; doi:10.1038/nrgastro.2012.241

The past year was highlighted by the completion of the human Immunochip project.[1] This international collaborative effort between 12 immune-mediated inflammatory disease groups—including Crohn's disease, ulcerative colitis, coeliac disease, type 1 diabetes mellitus, psoriasis, ankylosing spondylitis, multiple sclerosis, rheumatoid arthritis, IgA deficiency, autoimmune thyroid disease, primary biliary cirrhosis and systemic lupus erythematosus—involved the genotyping of 200,000 single nucleotide polymorphisms. The Immunochip project in IBD specifically aimed at fine mapping the 99 IBD loci identified in previous genome-wide association study meta-analyses and deep replication of the top 2,000 signals not followed up in these papers. An unprecedented total of 38,565 patients with IBD and 37,747 healthy individuals as controls were genotyped, and the number of confirmed genetic loci increased to 163. The majority of loci (110 of the 163; 67%) confer susceptibility to both Crohn's disease and ulcerative colitis, with only 30 loci thought to be specific for Crohn's disease and 23 specific for ulcerative colitis (although the majority of the risk alleles have the same direction of effect in the alternative phenotype). Almost 70% of the loci are shared with other immune-mediated inflammatory diseases, which is substantially higher than expected by chance. Remarkably, most variants are in noncoding regions and only 18% are in linkage disequilibrium with missense mutations. Furthermore, 40% of variants are in linkage disequilibrium with variants regulating gene expression. In terms of biology, the Immunochip project underscored the common susceptibility to IBD and infectious diseases, with several IBD genes implicated in Mendelian susceptibility to mycobacterial disorders (*IL12B*, *STAT1*, *IRF8*, *TYK2*, *STAT3* and *IFNGR2*) and leprosy (*NOD2*, *IL23R*, *TNFSF15*, *RIPK2*, *LRRK2* and *C13ORF31*). Functional experiments and sequencing efforts to identify causal variants are underway and will potentially explain how these genes trigger chronic inflammation in various organs.

Key advances

- The Immunochip project increased the total number of validated susceptibility loci for IBD to 163, with a strong contribution of genes also implicated in mycobacterial diseases and leprosy[1]
- Several new non-anti-TNF compounds have shown efficacy in phase III studies, amongst them tofacitinib for ulcerative colitis[4] and vedolizumab for ulcerative colitis[6] and Crohn's disease[7]
- Not all therapeutic approaches show efficacy across diseases; secukinumab, which showed encouraging results in psoriasis and rheumatoid arthritis, failed in Crohn's disease[8]
- In patients with acute severe colitis, ciclosporin is not superior to infliximab in inducing clinical response and avoiding colectomy after 3 months[3]

15 years of use and over 1 million patients treated has generated a large amount of safety and long-term efficacy data for anti-TNF agents. The drawbacks of TNF antagonist treatment are the loss of response caused by antibody formation and the costs associated with long-term administration of these drugs. Although many insights into understanding immunogenicity have been gathered, a number of initiatives have been launched investigating how to reduce costs. The STORI study[2] showed that patients in remission on combination treatment with azathioprine and infliximab could stop infliximab (and continue with azathioprine monotherapy); however, half of the patients will relapse within 1 year. Signs of subclinical inflammation at the time of stopping treatment (increased faecal calprotectin levels, sedimentation rate and C-reactive protein levels, or minor signs of endoscopic activity) are associated with increased risk of relapse. Along the same lines, a number of ongoing studies are investigating if prospective serum level monitoring and adaptation with drug escalation or de-escalation can reduce costs and improve care.

In acute severe colitis, the 1 million dollar question was whether anti-TNF agents should be preferred over ciclosporin to prevent patients from requiring colectomy. In a study by the GETAID group,[3] 115 patients with acute severe colitis not responding to intravenous steroids were randomly assigned to open-label intravenous ciclosporin or infliximab. The study was powered as a superiority study for ciclosporin. The primary end point was treatment failure defined as any of the following: absence of clinical response at day 7, relapse between day 7 and 98, absence of steroid-free remission at day 98, colectomy or death. The primary end point occurred in 60% of patients treated with ciclosporin and 54% of patients treated with infliximab. Both drugs, therefore, seem at least equally effective in patients who are naïve to azathioprine. As always, physicians need to balance efficacy with adverse effects and risk of opportunistic infections.

Several phase II and III studies of molecules targeting pathways other than TNF

were completed in 2012 (Supplementary Table 1 online). In ulcerative colitis, tofacitinib, an oral inhibitor of Janus kinases 1 and 3, showed dose-dependent efficacy and mucosal healing in a phase II study.[4] In this multicentre study, 194 patients with moderate-to-severe ulcerative colitis were randomly allocated to one of four doses of tofacitinib (0.5, 3, 10 or 15 mg twice daily) or placebo for 8 weeks. No concomitant immunomodulators or anti-TNF agents were allowed. A substantially higher proportion of patients receiving 15 mg tofacitinib twice daily showed clinical response (78%), clinical remission (41%) and endoscopic remission (27%) than patients on placebo (42%, 10% and 2%, respectively). The adverse effects most commonly observed were dose dependent (but reversible upon discontinuation) increases in LDL and HDL cholesterol levels, and infections. Two large phase III studies are ongoing to confirm these results and to study the efficacy of this drug in maintaining remission.

The efficacy of ustekinumab, a human monoclonal antibody targeting the p40 subunit of IL-12 and IL-23, was confirmed in a phase IIb study.[5] Expression studies and genetic data have underscored the importance of the IL-12/IL-23 pathway in patients with Crohn's disease, and a previous study with this compound had shown efficacy mainly in patients with anti-TNF resistance. Ustekinumab is approved for moderate-to-severe plaque psoriasis. The CERTIFI study[5] was a 36-week study including 8 weeks of induction treatment and 28 weeks of maintenance treatment in patients failing anti-TNF therapy. A total of 526 patients were randomly allocated to three doses of intravenous ustekinumab (1, 3 or 6 mg/kg) or placebo. The primary end point, clinical response at week 6, was significantly higher for the 6 mg/kg group than the placebo group (39.7% versus 23.5%, respectively; $P = 0.005$). Patients who responded to treatment were again randomized to receive subcutaneous ustekinumab 90 mg or placebo at weeks 8 and 16. At week 22, response (69.4%) and remission rates (41.7%) were considerably higher in the active group compared with the placebo group (42.5 and 27.4%, respectively). The safety profile was good, and only 0.7% of patients developed antibodies to ustekinumab by week 36.

Vedolizumab, a humanized antibody directed against α4β7 integrin, was studied in two phase III trials in ulcerative colitis and Crohn's disease, named GEMINI I and II, respectively.[6,7] Vedolizumab inhibits the binding of α4β7 integrin to its receptor MAdCAM-1, subsequently inhibiting homing of T cells to the gut. Both studies were integrated induction and maintenance studies and met their primary end points, although it should be noted that the results for ulcerative colitis were superior to those for Crohn's disease. At week 6, 47% of patients with ulcerative colitis treated with vedolizumab responded to treatment and 41% showed healing (in contrast to 25% for both end points in the placebo group);[6] the long-term results were even more impressive—45% of patients treated with vedolizumab every 4 weeks were in corticosteroid-free remission at week 52 (14% in the placebo arm) and 56% had mucosal healing (20% in the placebo arm). The corresponding figure for corticosteroid-free remission at week 52 in Crohn's disease was 28.8%.[7] If approved, vedolizumab will be the first drug that specifically targets inflammatory cell homing to the gut. The need to develop a gut-selective drug became clear after reports of progressive multifocal leucoencephalopathy with the non-gut-specific anti-α4 integrin natalizumab.

That not all anti-cytokine approaches are exchangeable across diseases was demonstrated in the proof-of-concept study with secukinumab, a human selective IL-17A monoclonal antibody, in 59 patients with moderate-to-severe Crohn's disease.[8] Phase II studies previously demonstrated efficacy and good safety with this compound in rheumatoid arthritis and psoriasis. The study in Crohn's disease was terminated prematurely after results showed not only lack of efficacy, but also more adverse events, especially infections, in patients treated with intravenous secukinumab (74% versus 50% in the placebo group). These results suggest that IL-17A might have protective functions in the gut. This protective effect was already indicated in the $CD45RB^{Hi}$ transfer model of colitis, in which disease aggravation was seen when T cells deficient in IL-17A and IL-17 receptor were transferred.[9] Interestingly, genetic variants in the IL-17 signalling pathway have also been associated with chronic candidiasis, which in part could explain why a number of serious fungal infections were observed in the Crohn's disease trial.[10]

Where will the new promising drugs be positioned if they become approved? From a clinical perspective, a large unmet need still exists in the therapeutic options for patients with IBD despite the advances delivered by anti-TNF agents. Not only do 10–15% of patients not respond to anti-TNF agents, 40% of patients lose response over time and need to switch to a second or even third anti-TNF agent. Even with earlier use of anti-TNF antibodies, one of five patients with ulcerative colitis and three of five patients with Crohn's disease still require colectomy or small bowel resections. The favourable safety profile of vedolizumab, and the stable response and remission rates over 52 weeks are true assets for the compound. However, loss of response to anti-TNF agents can occur well beyond a year, so longer follow-up data, including trough level and antibody data, are needed. Head-to head comparisons of the new agents with existing anti-TNF agents will advance the care of our patients and enable the best strategy for the individual to be selected. These studies need to be accompanied by molecular studies aiming to predict response to new anti-integrin approaches.

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Competing interests
S. Vermeire and P. Rutgeerts declare associations with Abbott, MSD, Pfizer, UCB Pharma. See the article online for full details of the relationships.

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Supplementary information
Supplementary information is linked to the online version of the paper at www.nature.com/nrgastro.

COLORECTAL CANCER DIAGNOSIS IN 2012

A new focus for CRC prevention—more serration, less inflammation

James E. East and Evelien Dekker

Knowledge of colorectal cancer (CRC) risks has been rebalanced in 2012. The 'serrated pathway' to CRC, exemplified by serrated polyposis syndrome, emphasizes the importance of serrated lesions. The dogma that patients with IBD are at high risk of CRC, however, might be overstated; optimizing CRC prevention needs to focus on patients at increased risk.

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Colorectal cancer (CRC) dominates gastrointestinal cancer prevention strategies, owing to its high prevalence and the accessibility and long dwell-time of the premalignant lesion. Although studies have confirmed the efficacy of interrupting the adenoma–carcinoma sequence to prevent CRC occurrence and death caused by CRC, the failure to effectively prevent right-sided CRC has been a concern.[1] Specifically, the adenoma–carcinoma sequence might not be the full story; in particular, in the right side of the colon a new 'serrated pathway' might have an important role (Figure 1).[2]

Hyperplastic polyps were thought to have no malignant potential but molecular and pathological evidence now indicates that a subset of these lesions—sessile serrated polyps (SSPs)—might account for 20–30% of CRCs.[2] SSPs are most common in the right side of the colon and in females. Although previously thought to be rare in the proximal colon, Kahi *et al.*[3] revealed a range of polyp detection rates amongst 15 endoscopists of 1–18% for proximal serrated polyps, with a clear correlation ($r = 0.71$–0.73, $P < 0.004$) between serrated polyp detection and adenoma detection. When compared with the adenoma detection rates recommended in the US surveillance guidelines (25% for men, 15% for women), the equivalent serrated polyp detection rate was 5% for both sexes.[3] This detection rate could be a suitable benchmark for endoscopists to aim for when assessing if sufficient numbers are being detected. The level of detection that correlates with effective right-sided CRC prevention is not yet known.

The importance of serrated polyp detection has also been shown by epidemiological cohort studies of patients with serrated polyposis syndrome (SPS) from Europe and the USA. Patients with >20 serrated polyps throughout the colon or ≥5 serrated polyps proximal to the rectosigmoid with two or more ≥10 mm in size meet WHO 2010 criteria for the syndrome,[4] as do first-degree relatives of these patients with at least one proximal serrated polyp. These patients are at substantially increased risk of CRC. Indeed, a cohort of 44 patients with SPS had a relentless development of new colorectal neoplasia whilst under surveillance, including two carcinomas.[4] No evidence of polyposis or dysplasia was found in the upper gastrointestinal tract in a subset of 22 patients who underwent surveillance using gastroscopy, although this series was very small.[4] The long-term risk of CRC in SPS could be as high as 7% at 5 years during surveillance with white-light colonoscopy.[5] First-degree relatives have a relative risk of five for CRC when compared with the general population and a risk of SPS of 39.[4]

Key advances

- Serrated polyps are premalignant lesions that need to be detected and resected;[4] surveillance intervals have now been changed in new US guidelines[6]
- Unsuspected incomplete snare polypectomy is more common than previously thought and might contribute to interval cancers after 'clearing' colonoscopy[7]
- Colorectal cancer incidence in patients with colitis seems to be much lower than previously estimated; surveillance, therefore, might be better focussed on higher-risk patients[9,10]

The importance of sporadic serrated polyps and of SPS has led to a change in the 2012 update to US multisociety polyp follow-up guidelines.[6] Now, patients with SPS are recommended to have yearly colonoscopic surveillance, and those with SSPs ≥10 mm in size, SSPs with dysplasia or traditional serrated adenomas to have surveillance at 3 years. Patients with SSPs <10 mm in size should have surveillance at 5 years.[6] European guidelines do not reflect these criteria, but updates are likely to follow.

Failure to prevent right-sided CRC might not be the sole result of a failure to appreciate the importance of serrated lesions or flat adenomas. Other potential causes include rapidly growing lesions or incomplete polypectomy. Interval cancers are more common at sites where a polypectomy was previously performed. Snare polypectomy has been assumed to be a comprehensive technique for complete polyp excision, unlike hot biopsy which leaves residual viable tissue in an important subset of cases. Concerning data were reported this year in the Complete Adenoma REsection (CARE) study in which, directly after having performed snare resection of a polyp, endoscopists removed a biopsy sample from the lesion edge to determine if histologically apparent residual neoplasia was present.[7] In 10.1% of resections polyp remnants were found, with high rates for larger polyps (17%, 10–20 mm lesions), and serrated polyps (31%), where the lesion edges can be hard to define.[7] A *post hoc* analysis suggested marked differences of up to 3.4-fold between endoscopists in their rates of residual polyp remnants.[7] These findings dovetail with known epidemiological data to explain why interval cancers might occur. Further work is needed to refine polypectomy technique and re-educate practicing endoscopists to reduce this risk. Recommendations to try to incorporate a small visible rim of normal tissue when performing polypectomy might be advisable.

In contrast to the concerns about sporadic and syndromic CRC via new serrated pathways, new scrutiny of the risk of CRC

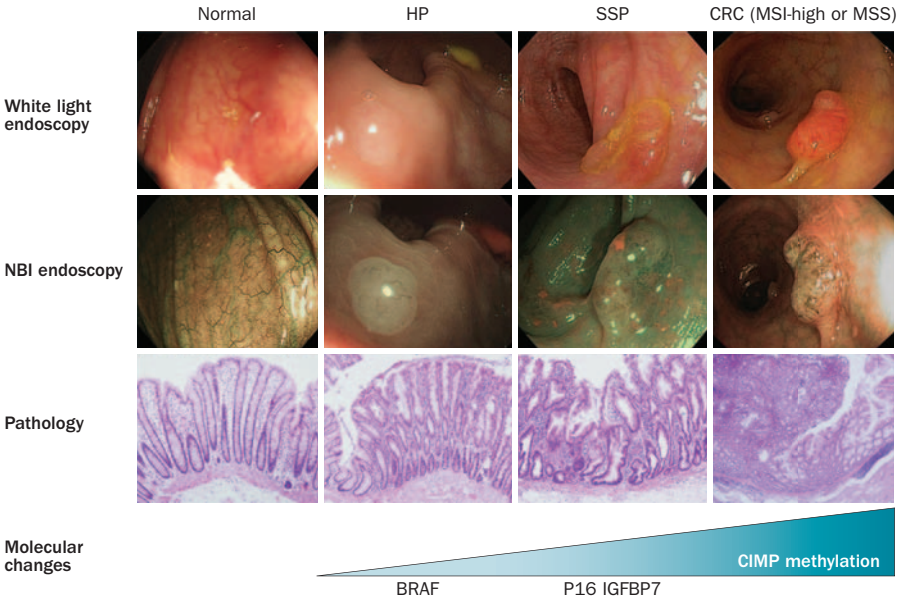

Figure 1 | Endoscopic, pathological and molecular changes for the 'serrated pathway' to CRC from normal mucosa to SSP and carcinoma. Paired endoscopic white-light and NBI images of representative lesions are shown; advanced endoscopic imaging techniques might help to detect serrated polyps. SSPs are thought to be the principal precursor lesion of CRCs arising by the serrated pathway; whether SSPs develop from pre-existing hyperplastic polyps or directly from normal mucosa remains unclear. A point mutation in BRAF (V600E) results in enhanced cell proliferation by inhibition of apoptosis of colonic epithelial cells. Further progression is most likely caused by methylation of CIMP regions resulting in epigenetic silencing of multiple genes.[2] The far right pathological image shows a CRC adjacent to an SSP. Abbreviations: CIMP, CpG island methylation of multiple genes; CRC, colorectal carcinoma; HP, hyperplastic polyp; MSI, microsatellite instablity; MSS, microsatellite stable; NBI, narrow-band imaging; SSP, sessile serrated polyp.

in IBD suggests it is lower than expected. Historical estimates by meta-analysis reported an 18% risk of CRC at 30 years of disease;[8] many of the studies included were from academic centres that might overestimate risk by over-representing patients with severe disease. Two population-based estimates from this year suggest that the risk is reduced and might be decreasing.[9,10]

In a Danish whole-population-based study, the risk of CRC had decreased over the past 30 years for patients with ulcerative colitis with no increased risk in modern (1999–2008) cohorts.[9] For Crohn's colitis, the risk was below the population average (0.85, 95% CI 0.67–1.07). Why the risk has reduced is unclear, but might reflect better control of inflammation through aggressive medical therapy with immunomodulators. Endoscopic surveillance is rare in Denmark and the colectomy rate has remained steady. Patients with primary sclerosing cholangitis (PSC) and ulcerative colitis had a ninefold increased relative risk of carcinoma compared with those without PSC, although these cases only accounted for 8 of 268 (3%) cases in the cohort.[9] Young age at ulcerative colitis onset was associated with an increased risk versus the rest of the population (43-fold increased relative risk for ages 0–19 years at diagnosis, and 2.4-fold for ages 20–39 years). These age groups accounted for 61 of the 268 cancer cases. Therefore, classic risk factors for dysplasia and cancer (PSC and diagnosis at a young age) might still identify high-risk patients even in population-based cohorts.

The risk of CRC in the IBD proportion of a US population was only 60% above the population average.[10] Increased rates of CRC were noted in patients with Crohn's disease and ulcerative colitis. During 1998–2010, a trend towards reduced rates of CRC was not observed despite a small increase in immunomodulator use and a surveillance colonoscopy programme. The rates are still modestly elevated compared with the five-fold increases in risk reported in the 1980–1990s.[10] Such a low rate overall suggests that additional surveillance of all patients with colitis beyond population screening is not needed. Updated UK guidelines[8] recommend an approach based on risk factors, which might find international favour.

Looking forward, we need to be meticulous in our withdrawal technique during colonoscopy to detect serrated polyps, and during polypectomy to remove them. Perhaps we should also redirect our focus from all patients with longstanding IBD towards those at higher risk.

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LIVER FIBROSIS IN 2012

Convergent pathways that cause hepatic fibrosis in NASH

Scott L. Friedman

Efforts to understand fatty liver disease have focused on the gut microbiome's stimulation of hepatic injury and fibrosis through specialized signalling complexes at the cell surface and in the cytosol of liver cells. Combined with increased hedgehog activity and progenitor cell expansion, new clues are emerging to elucidate the pathogenesis of fibrosis.

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NAFLD associated with obesity and metabolic syndrome often leads to NASH, which is a condition marked by hepatic inflammation and risk of advanced fibrosis.[1] The prevalences of NAFLD and NASH are increasing at a staggering rate worldwide, and in the next decade will exceed viral hepatitis as a cause of end-stage liver disease to become the primary indication for liver transplantation. The trend is especially alarming among children as rates of paediatric obesity soar.[2] With this looming health crisis, efforts to explain the underlying causes of fibrosis have taken on a new urgency, yielding heartening progress. At least two convergent pathways are now implicated in NASH-associated fibrosis: inflammasome activation and its interaction with the microbiome; and progenitor cell amplification associated with increased levels of hedgehog signalling (Figure 1). These concepts are nicely illustrated in several publications from 2012.

The inflammasome is a multiprotein scaffold and key mediator of the innate immune response within the cytosol of immune and nonimmune cells.[3] Inflammasome activation leads to cleavage of caspase-1, which culminates in the secretion of the inflammatory cytokines IL-1β and IL-18. Inflammasomes are classified by the type of pattern-recognition receptors (PRRs) they contain, which are specific for different external stimuli, typically bacteria or viruses. Thus, within liver cells, the inflammasome can mediate the cellular response to bacterial fragments derived from the gut microbiota following their transport from the intestinal lumen through the portal circulation.

A growing number of publications have implicated the inflammasome in the pathogenesis of NASH, and therefore indirectly to the pathogenesis of fibrosis, given that inflammation associated with NASH is profibrotic. An elegant study in mice by Henao-Mejia and colleagues directly links the gut microbiota composition to specific PRRs of the inflammasome, and to the development of NASH histology.[4] Mice genetically deficient in any of three inflammasome components *Casp1*, *Nlrp3* (*NOD-like receptor family, pyrin domain containing 3*) or *Asc* (*apoptosis-associated speck-like protein containing a carboxy-terminal CARD*; also known as *Pycard*), developed more hepatic inflammation and increased serum levels of alanine aminotransferase and aspartate aminotransferase than control, wild-type mice when fed a methionine–choline diet, which induces fatty liver. Deficiency in these components led to altered composition of the colonic bacterial flora, which could be transmitted to healthy wild-type mice when the two strains were housed together, leading to NASH-like liver histology in the healthy animals when fed a high-fat diet. The investigators further linked this altered microbiome to activation of Toll-like receptors (TLRs) 4 and 9 within the liver; mice deficient in either of these cell surface receptors had reduced hepatic injury (as well as attenuated TNF signalling, which is downstream of both TLRs) when challenged with a high-fat diet.

These findings illuminate the well-regulated choreography between the gut and liver that is dependent on the composition of the microbiota, and reinforce previous reports implicating the inflammasome in this interaction.

Building on this study, a report by Miura and colleagues[5] links the inflammasome to fibrosis more directly in an experimental model in mice that lack *Tlr2* (like TLR4 and TLR9, TLR2 is a cell surface receptor that signals to the inflammasome). In this study, the investigators demonstrated that TLR2 signalling by hepatic macrophages (Kupffer cells) is provoked by palmitic acid, which is an abundant free fatty acid in patients with NAFLD. Mice lacking *Tlr2* had reduced inflammasome activation, with less inflammation and fibrosis when fed a diet that induced fatty liver.

What do these new insights mean for patients with NASH and hepatic fibrosis? For one thing, they begin to firmly establish the role of the gut microbiota in contributing to the pathogenesis of NASH and fibrosis, which in turn could help explain why the prevalence of metabolic syndrome and

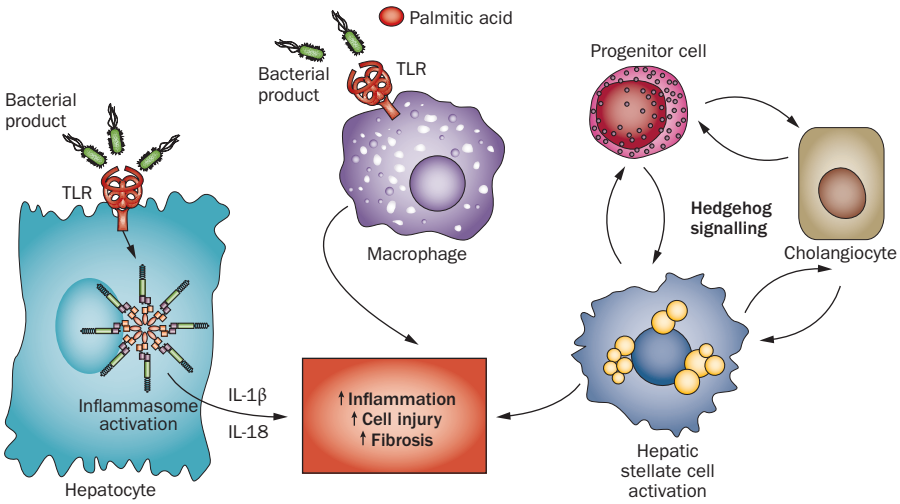

Figure 1 | Emerging pathways of fibrogenesis in fatty liver disease. Studies indicate that at least two pathways link the pathogenesis of fatty liver disease to hepatic fibrosis. The first requires activation of the inflammasome, an intracellular protein scaffold that generates IL-1β and IL-18, in both hepatocytes and hepatic macrophages, thereby promoting inflammation and cell injury. The second, hedgehog signalling, involves crosstalk between progenitor cells, cholangiocytes (bile duct epithelial cells) and activated hepatic stellate cells, which either generate hedgehog ligands and/or express its receptors to stimulate fibrosis by hepatic stellate cells.

Key advances

- An intracellular protein complex, called the inflammasome, in liver cells is implicated in the pathogenesis of NASH by transducing signals that originate from gut-derived bacteria[4,5]
- Altered composition of the gut microbiota might contribute to NASH pathogenesis, leading to increased hepatic injury and fibrosis[4,5]
- Progressive fibrosis in NASH is associated with increased activity of hedgehog signalling in the liver, in conjunction with the appearance of cells that express progenitor cell markers[7,9]

fatty liver has accelerated so precipitously in less than a generation. It is increasingly likely that broad changes in our microbiome composition—perhaps influenced by diet, environment, genetics or antibiotic usage—have created this relatively new disease.[6] Second, they suggest that new therapeutic avenues might attempt to alter the microbiota in an effort to attenuate the chronic inflammation associated with metabolic syndrome and fatty liver, perhaps using probiotics or narrow-spectrum antibiotics. Given the tremendous number of bacteria that inhabit the human colon, it is remarkable that their contribution to health and disease has been overlooked for so long.

Turning to human rather than experimental NASH, two related studies have used immunohistochemistry of liver biopsy specimens to implicate hedgehog signalling and progenitor cell activation in the pathogenesis of NASH fibrosis. The first study from Guy and colleagues[7] exploits the availability of a large number of biopsy samples generated by the NIH NASH Clinical Research Network to assess the relative activity of the hedgehog pathway. This molecule was initially identified as a developmental morphogen, but is now also implicated in adult diseases, especially cancer.[8] Staining for sonic hedgehog protein, one of three major hedgehog ligands in vertebrates, was increased in portal cells (especially bile duct cells) and was correlated with fibrosis stage and hepatocyte ballooning, based on the well-validated NASH histologic scoring system. Concurrently, expression of GLI2 (a hedgehog-regulated target gene) was increased in cells that also expressed K7 (also called CK7), which is thought to mark a subpopulation of epithelial progenitors. GLI2 was also expressed in stromal cells that expressed vimentin. These findings suggest that increased hedgehog signalling, derived from bile ductular cells and acting upon progenitor and stromal cells (including activated stellate cells), somehow promotes fibrosis, but the mechanism is not fully clarified.

In a complementary study, Nobili and colleagues[9] used CK7 staining to examine the hepatic progenitor response in 30 children with NAFLD. The number of hepatic progenitor cells correlated with the degree of fibrosis in these patients,[9] and the progenitor cells also expressed the adipogenic hormones adiponectin, resistin and glucagon-like peptide 1. These findings are similar to previous studies implicating the 'ductular reaction', which involves progenitor cell expansion in hepatic fibrosis.

Together these two descriptive studies by Guy *et al.*[7] and Nobili *et al.*[9] begin to implicate progenitor cells in the pathogenesis of fibrosis associated with NASH, but the findings have not yet crystallized into a coherent mechanism, and many questions remain. From which cells are progenitors derived and what is their relationship to stromal cells? Do progenitor cells arise because of a replication block in hepatocytes, or from other signals? Do progenitor cells stimulate fibrosis, and if so how? Is hedgehog signalling a driver in this progenitor cell and/or fibrotic response, and if not, what are the other signals that promote fibrosis linked to progenitor cell expansion? Now that the features of the human disease are being clarified, further analyses using either comprehensive genomic analysis of these human tissues or genetically altered mouse models will further refine an interesting, but as yet hazy, picture.

In aggregate, the above studies illustrate the intensifying interest in understanding fibrosis in NAFLD and NASH, a clinical problem that is certain to cause rising morbidity and mortality unless new therapeutic prospects emerge.

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Competing interests
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GUT MICROBIOTA IN 2012

Toward understanding and manipulating the gut microbiota

Jesse D. Aitken and Andrew T. Gewirtz

New techniques have introduced unprecedented sensitivity to the investigation of the gut microbiota, enabling insights into the discrete contributions of select bacterial species and advancing our mechanistic appreciation of the roles of diet and host immunity in limiting or enabling metabolic and inflammatory disease.

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The human intestinal tract is inhabited by ~100 trillion bacteria, comprising at least 3,000 distinct species—the gut microbiota. This diverse microbial community has an important role in metabolism and immunity and is increasingly appreciated as a

central 'environmental factor' in numerous diseases, including IBD, metabolic syndrome and cancer. As such, manipulation of the gut microbiota could represent a novel means to treat these increasingly common inflammatory diseases. Herein, we review some of the key areas of progress in this endeavour in 2012.

Previous studies have shown that changes in microbiota composition correlate with development of deleterious inflammatory and metabolic phenotypes that can be transferred upon microbiota transplant. Such work has fostered a phenomenological appreciation of the microbiota's influence, but advances in analytical chemistry (especially nucleic acid sequencing technologies) have enabled interrogation of species-level contributions to the microbiota ecosystem and overall host health; a striking example is the demonstration that a diet containing only milk-derived fats promotes dramatic expansion of *Bilophila wadsworthia* (a sulphite-reducing bacteria), owing to a milk-fat-induced increase in biliary taurine.[1] This bloom of a normally undetectable bacterial species was accompanied by an enhanced T-helper-1 immune response, induced colitis in *Il10*-deficient mice and conferred sensitivity to induction of colitis in wild-type mice. This work is the first (of probably many) to report specific diet–host–microbe interactions capable of causing dysbiosis that interlink metabolic and inflammatory changes.

States of chronic inflammation (colitis) have long been associated with cancer, although mechanistic links remain poorly defined. Although inflammation-associated oxidant production was presumed to be the major means of inducing DNA damage—the key driver of carcinogenesis—findings from 2012 reveal that such damage is also exacerbated by genotoxin-producing *Escherichia coli*.[2] Such bacteria increased in relative abundance after the initiation of inflammation and were associated with colon cancer in both humans and mouse models. This work underscores the multifactorial and interrelated nature of microbiota-mediated disease as inflammation promotes DNA damage primarily and by facilitating the growth of genotoxic bacteria.

Development of host–microbiota symbiosis is perhaps the most ancient and central evolutionary process; proper management of the microbiota is essential for basic daily survival. Indeed, considering that the gut represents a roughly tennis-court-sized interface with the outside world, immunity and metabolism are, by necessity, tightly intertwined. Loss of B-cell-derived IgA results in a dysregulated microbiota that requires a compensatory immune response from intestinal epithelial cells.[3] This shift away from normal Gata4-mediated metabolic function in favour of enhanced interferon-inducible immune pathways results in undernourishment (specifically impaired lipid absorption) with gene expression resembling that seen in immunodeficient and HIV-positive humans.[3] The opposite scenario, that of malnutrition causing dysbiosis, has now been mechanistically established. Mice lacking angiotensin 1 converting enzyme 2 have impaired absorption of neutral amino acids (including tryptophan), resulting in colitis and diarrhoea that mimics the human disease pellagra.[4] Such malabsorption also caused dysbiosis via mTOR-mediated reduction in antimicrobial peptide secretion in the small intestine, which could be rescued with supplementation with tryptophan or the metabolic end product nicotinamide. This work suggests that a variety of metabolic deficiencies feature, at least in part, dysregulation of the microbiota and might thereby be ameliorated through careful manipulation of resident bacteria and/or immunity.

The treatment of overnutrition, however, is of greater interest to the developed world, which faces an epidemic of metabolic syndrome related to increased consumption and reduced physical activity. Metabolic syndrome is promoted by loss of inflammasome signalling. Deletion of *Nlrp3* or *Nlrp6* in mice results in an altered microbiota capable of exacerbating the development of metabolic syndrome via increased levels of Toll-like receptor (TLR)4 and 9 ligands;[5] specifically, increased levels of such ligands in portal circulation promoted TNF-mediated NAFLD development. Interestingly, simply co-housing these immunocompromised mice with wild-type animals conferred the observed predispositions to NAFLD and obesity, with the implication that an altered pathogenic microbiota might be somewhat analogous to an infectious disease. Whilst acknowledging the important caveats of limited study time and use of coprophagic rodents, a similar process could be occurring in humans, especially in family groups cohabitating for extended periods.

A study of *Tlr5*-deficient mice indicates that the state of microbiota flux itself might promote inflammation and dysmetabolism in general rather than the presence or absence of particular bacterial species or genes.[6] Some *Tlr5*-deficient mice develop colitis whereas others develop low-grade inflammation and metabolic syndrome without colitis and, whilst overall penetrance is stable from generation to generation, the development of colitis occurs randomly. Weekly sampling of faecal bacteria revealed that, unlike wild-type mice, *Tlr5*-deficient mice had increased week-to-week changes to the microbiota and that such volatility was observed whether or not mice eventually developed colitis. This phenomenon, wherein the host must engender a compensatory response to attempt to control microbiota composition, could reflect human IBD, which is promoted by disparate innate immune deficiencies but in which at least half of overall disease risk is dictated by nongenetic (environmental) factors. Furthermore, in these mice, transient colonization by normally undetectable species—a human Crohn's-disease-associated *E. coli* strain—promoted chronic inflammation and colitis that persisted well after clearance, suggesting that uncontrolled volatility could promote inflammation simply by allowing deleterious species the opportunity to interact with the host. Studies in humans, though enlightening, would require long-term repeat sampling starting well before IBD develops.

Given that the increasing prevalence of IBD and obesity has occurred recently in evolutionary terms, the contribution of host genetics to such population-level microbiota changes is probably fairly minor. Rather, societal and technological progress has reoriented the host–microbiota axis. One possible culprit is the widespread use of antibiotics by both humans and large-scale farming operations that have routinely used low-dose antibiotics to stimulate weight gain

Key advances

- Changes in diet can facilitate the expansion of deleterious (formerly niche) bacterial species that can induce inflammation[1]
- Mice with a specific immunodeficiency develop metabolic syndrome (including NAFLD) and promote similar disease in wild-type mice when co-housed[5]
- Administration of low-dose antibiotics to mice induces adiposity and alterations to the microbiota that resemble those seen in metabolic syndrome[7]
- In humans, clinical infusion of bacteria from lean donors ameliorates insulin resistance in recipients with metabolic syndrome[9]

for >50 years. Young mice exposed to single or combination antibiotic therapy exhibit lasting increases in adiposity and accelerated bone mineral deposition; whilst total bacterial numbers remained similar to untreated mice, the composition of the microbiota was altered substantially.[7] Notably, expansion of the phylum Firmicutes and contraction of Bacteriodetes, a shift that generally accompanies weight gain in a variety of models, was observed.[7] Furthermore, bacterial expression of genes related to carbohydrate metabolism was altered, resulting in increased energy extraction and hepatic lipogenesis.[7]

Interestingly, changes in the microbiota that promote adiposity could have a normal physiological role, to increase energy extracted from food throughout pregnancy and lactation.[8] Indeed, by delivery, pregnant women develop many of the hallmarks of metabolic syndrome: adiposity; insulin resistance; increased bacterial load with reduced diversity; and an altered Firmicutes:Bacteriodetes ratio. Moreover, microbiota from a third trimester woman induced a similar metabolic phenotype when transferred to germ-free mice.[8] These data suggest that metabolic syndrome mimics, or even co-opts, evolved mechanisms designed to increase energy extraction during the most metabolically demanding phases of reproduction.

Despite our basic understanding of the specific mechanisms underlying metabolic syndrome, treatment avenues do exist. A well-controlled clinical trial designed to manipulate the microbiota to treat insulin resistance (a hallmark of metabolic syndrome) achieved remarkable success.[9] Small intestinal infusion of gut bacteria from lean donors increased sensitivity to insulin within 6 weeks without changes in diet, hormone profile or total number of bacteria; controls given their own microbiota showed no improvement. Consistent with experimental work, an increase in microbiota diversity, with notable expansion of butyrate-producing bacteria, was observed. Thus, although still in very early stages, the development of techniques that replace or augment the host's microbiota hold notable promise for the treatment of metabolic disease and could be extended to treat any number of gut-linked disorders (including IBD). Indeed, increasingly fine techniques that enable interrogation and manipulation of the microbiota might presage next-generation treatment regimes that are both personalized and act through existing natural pathways.

Research from 2012 has begun to reveal some of the specific molecular mechanisms by which diet and host immunity determine microbiota composition. Researchers have also made substantial headway in understanding how specific bacteria contribute to a broad array of intestinal disease states. An emerging central theme is that host defence and metabolism are highly intertwined with innate immunity and inflammation, serving as a key interface of these crucial biological processes. 2012 has also seen major progress in the identification and examination of societal factors that have eroded the established distinction between commensal and pathogen and established a nuanced relationship between host and symbiont, as well as proof that interventional therapy represents an avenue by which to reassert host control over the microbiota. Given the increasing appreciation of the relevance of gut bacteria in host health, methods of establishing and maintaining such control holds great promise as treatment for a variety of heretofore intractable chronic intestinal diseases.

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LIVER TRANSPLANTATION IN 2012

Transplantation for liver cancer—more with better results

Chung-Mau Lo

Liver cancer remains an evolving indication for liver transplantation in the year 2012 as advances are made in patient selection, neoadjuvant treatment and living-donor liver transplantation. Patient survival is improving and, as patient selection and treatment advances, more transplantations can be conducted on patients with liver cancer.

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The year 2012 marks half a century since the historic first attempt of human liver transplantation by Thomas Starzl in 1963. Liver transplantation has since evolved from an experimental procedure used as a last resort to a highly successful, life-saving procedure with 1-year and 5-year survival rates that exceed 85% and 70%, respectively. Liver cancer was one of the first indications for liver transplantation (Figure 1), but it remains controversial owing to the risk of recurrence and inferior outcome in the context of worldwide organ shortage. The European Liver Transplant Registry (ELTR) showed substantial changes in the proportion of liver cancers as an indication for liver transplantation, from 50% in the 1980s to only 10% in the 1990s.[1] This indication has increased in the past 10 years in a linear fashion from 10% to 20%. The 1-year survival rate has also increased (by 36%) during this time, which is attributed

to improvements in patient selection rather than in treatment. Here, we highlight several studies that might improve the outcome further and also widen the application of liver transplantation for liver cancer.

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and a common indication for liver transplantation. The improvement in outcome and the increase in the proportion of liver transplantations for liver cancer in the past 15 years are primarily related to the adoption of the Milan criteria (1 tumour ≤5 cm or 2–3 tumours ≤3 cm, and no evidence of vascular invasion or extrahepatic metastasis), first published in 1996.[1] Evidence now suggests, however, that the metastatic potential of a cancer is inherent in its biologic behaviour and might not be related to its size and number. An international consensus conference on liver transplantation for HCC recommended that the Milan criteria should remain as the benchmark for selection or prioritization of patients, but serum α-fetoprotein (AFP) as a serum biomarker might add prognostic information to the imaging criteria.[2]

The Liver Transplantation French Study Group identified serum AFP, tumour number and tumour size as three independent prognostic factors, which were incorporated into a model to predict recurrence after transplantation.[3] A score was calculated by adding the individual points for each of the three variables and a cut-off value of two separated patients with high (score >2) and low (score ≤2) risk of recurrence with 5-year recurrence rates of 50.6% and 8.8%, respectively.[3] The AFP model has prognostic value in subgroups of patients both within and beyond the Milan criteria. Net reclassification showed that, in patients beyond the Milan criteria, as much as 74% of those who did not experience recurrence would have been classified as low risk by the AFP model and, hence, would be acceptable for transplantation.[3] Of note, the AFP model is applicable to patients undergoing reassessment during the waiting phase for further selection after down-staging or drop-out as a result of tumour progression. Serum AFP is a simple and reliable biomarker that provides repeatable results and should be included in the clinical decision-making in selecting patients with HCC for liver transplantation.

MicroRNA (miRNA) expression profile might also enable the prediction of tumour biology. A panel of 67 miRNAs in liver tumour tissue when used as a biomarker identified 9 of 12 patients within Milan criteria whose HCC recurred and eight of 11 patients beyond Milan criteria who did not have recurrence.[4] Although this biologic matrix might enhance the accuracy of the Milan criteria, the advantage of such a molecular study of gene expression over the use of conventional histopathological features (such as microvascular invasion or cellular differentiation) remains to be defined. Moreover, obtaining the appropriate amount of tissue by needle biopsy for miRNA amplification and biomarker testing is logistically difficult, if not impossible. The risks posed by biopsy, particularly for patients with decompensated cirrhosis with coagulopathy, portal hypertension and ascites is considerable. Hence, the potential value of incorporating tissue biomarkers as one of the selection criteria is limited, except in the strategy of initial liver resection before salvage transplantation for recurrence. Fuks *et al.*[5] showed that such a strategy resulted in similar survival when compared with primary transplantation performed on an intention-to-treat basis. Nonetheless, many patients who have recurrence after liver resection might not be eligible for transplantation[5] and salvage liver transplantation has been shown to be a strong predictor of recurrence, even more so than serum AFP and microvascular invasion.[6] Examination of the liver resection specimen for histology and biomarkers has the potential to provide valuable information that could guide clinical decisions towards earlier salvage transplantation in the absence of recurrence or against transplantation after recurrence.

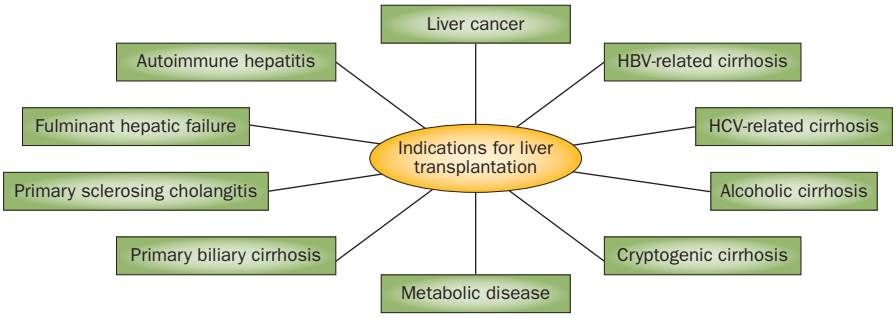

Figure 1 | Common indications for liver transplantation.

Hilar cholangiocarcinoma is the second most common primary liver cancer. Most patients present with locally advanced unresectable disease and, even when resection is possible, recurrence rate is high. Initial enthusiasm to adopt liver transplantation to overcome the limitations of resection was hindered by poor patient outcome. Hence, hilar cholangiocarcinoma was regarded as a contraindication to liver transplantation until a protocol of neoadjuvant chemoradiation before liver transplantation reported encouraging results. Since June 2009, the United Network of Organ Sharing/Organ Procurement and Transplantation Network (UNOS/OPTN) approved the allocation of Model of End-Stage Liver Disease (MELD) exception score to patients who have completed an approved neoadjuvant therapy protocol.[7] The MELD exception score was arbitrarily set equal to the current assigned score for HCC, representing an estimated 10% rise in waitlist mortality every 3 months.

A US multicentre study demonstrated that neoadjuvant chemoradiotherapy before liver transplantation in patients with unresectable hilar cholangiocarcinoma was effective, providing justification for prioritization in organ allocation by assigning MELD exception to these patients.[7] Of the 287 patients, 71 (25%) dropped out after a median of 4.6 months; the average drop-out rate of 11.5% every 3 months supports the current UNOS/OPTN policy of MELD exception allocation. Of the 214 patients who underwent liver transplantation after neoadjuvant therapy, the 5-year disease-free survival rate was 65%, comparable to the outcome of liver transplantation for other malignant or nonmalignant liver diseases.[7] The UNOS/OPTN criteria for MELD exception (mass >3 cm, metastatic disease at transplantation or direct transperitoneal tumour biopsy) was the only statistically significant predictor of outcome (HR 2.98). Patients who fulfilled the criteria had a 5-year disease-free survival of 72%.[7] This study represents the best available evidence of the efficacy of neoadjuvant therapy for hilar cholangiocarcinoma and supports accepting and prioritizing these patients for liver transplantation. Randomized trials in patients with hilar cholangiocarcinoma are almost

Key advances

- Serum α-fetoprotein as a circulating biomarker enhances the accuracy in predicting recurrence after liver transplantation for hepatocellular carcinoma[3]
- Tissue biomarkers based on molecular studies of gene expression correlate with tumour biology
- Neoadjuvant chemoradiation before liver transplantation in patients with unresectable hilar cholangiocarcinoma can achieve good survival and justify their prioritization in organ allocation[7]
- Increases in donor safety and a wider application of left-lobe living-donor liver transplantation might increase the number of patients with liver cancer who benefit from transplantation[9,10]

impossible owing to the rarity of the disease and ethical consideration.

As we continue to improve the outcome and extend the benefit of liver transplantation to a greater number of patients with liver cancer, the issue of deceased donor organ shortage worsens because liver transplantation is a zero-sum game (whereby the gains in liver transplantation in one patient currently equal the losses in another). Living-donor liver transplantation (LDLT) provides an unlimited source of liver grafts and could transform liver transplantation into a nonzero-sum game. Early access to LDLT would enable drop-outs resulting from tumour progression or liver failure caused by complications of neoadjuvant treatment to be avoided. In patients who underwent neoadjuvant therapy for hilar cholangiocarcinoma, 29% of the transplantations were from living donors,[7] which was much higher than the overall proportion of LDLT in the USA (<5%).[8] Two studies from Asia found that left-liver donation is safer than right-liver donation and can achieve excellent outcome in adult recipients.[9,10] For every 5% reduction in minimum size of graft required (potentially realized with advances in surgical skill, adoption of portal flow modulation and improved postoperative care to prevent small-for-size syndrome), the number of left-liver transplantations could be doubled,[9] resulting in a reduced risk for donors.

The results of liver transplantation for liver cancer are improving through improved patient selection and innovation in neoadjuvant therapy. Better donor safety and a wider application of left-lobe LDLT might enable higher numbers of patients to benefit from transplantation.

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HEPATITIS C IN 2012

On the fast track towards IFN-free therapy for hepatitis C?

Heiner Wedemeyer

With the first HCV protease inhibitors approved in 2011, we are currently in a transition phase towards a shift in treatment paradigm. Within the next 3 years, the vast majority of patients with hepatitis C will probably be treated with completely different drugs in most Western countries.

Wedemeyer, H. *Nat. Rev. Gastroenterol. Hepatol.* **10**, 76–78 (2013); published online 8 January 2013; doi:10.1038/nrgastro.2012.247

The field of hepatitis C therapy is rapidly evolving. In light of this changing landscape, treating patients with hepatitis C in 2013 will be challenging. Several questions are currently under debate.[1] Which patients should be treated now with the available compounds? Which patients can wait for IFN-free treatments? When can we expect these IFN-free therapies and will the new drugs have good efficacy and tolerability? What will be the cost of IFN-free therapy? Are there still alternative options to improve efficacy of currently used drugs?

For more than two decades, IFN-α has been the basis of antiviral therapies for chronic hepatitis C, leading to sustained virologic response (SVR) rates of 30–90% depending on the HCV genotype, stage of liver disease and host genetics.[2] However, therapies containing IFN are associated with a wide spectrum of adverse events and thus only a minority of HCV-infected individuals have been treated with IFN-α.[3] In contrast to most other persistent viral infections, a cure for HCV infection is possible. HCV has a purely cytosolic life cycle and potent suppression of viral replication in the absence of resistance can cure HCV-infected cells. An obvious approach to improve therapy of hepatitis C was therefore to combine novel direct-acting antivirals (DAAs) that target different steps in the HCV life cycle.

Indeed, the first proof-of-concept study demonstrating SVR in patients with chronic hepatitis C with IFN-free DAA combination therapy was published in 2012.[4] The compounds investigated in this study were the HCV nonstructural protein 5A inhibitor daclatasvir and the HCV nonstructural

protein 3 protease inhibitor asunaprevir. Patients infected with HCV genotype 1a or 1b who were null responders to a previous course of antiviral therapy based on IFN-α received a 24-week course of either a quadruple therapy of PEG-IFN-α2a–ribavirin, daclatasvir and asunaprevir or daclatasvir and asunaprevir without PEG-IFN-α–ribavirin. Quadruple therapy led to cure of HCV infection in all 10 patients treated. This finding is remarkable considering the low cure rate in null responders treated with PEG-IFN-α, ribavirin and telaprevir or boceprevir. IFN-free therapy with daclatasvir and asunaprevir induced a rapid decline in HCV RNA in all patients and an SVR was observed in 4 of 11 individuals, with both genotype 1b-infected patients clearing HCV. Therapy with daclatasvir and asunaprevir was also highly successful in a study performed in Japan that only enrolled patients infected with HCV genotype 1b.[5] All 10 patients who had not responded to a previous course of PEG-IFN-α–ribavirin therapy achieved SVR with daclatasvir and asunaprevir.

Various other IFN-free combinations of DAAs are currently in clinical development (Figure 1). Data presented during the past 12 months have highlighted that it is not sufficient to simply combine different DAA classes, but that how potent an antiviral is and whether it has barriers to resistance are key factors in preventing treatment failure. A study by Zeuzem *et al.*[6] investigated the antiviral activity of the HCV protease inhibitor GS-9256 and the non-nucleoside polymerase inhibitor tegobuvir over 28 days. Tegobuvir–GS-9256 led to a pronounced decline in levels of HCV RNA during the first 48 h of therapy, but a viral rebound was observed in most patients after day 7. Only 1 of 15 patients maintained virologic suppression until day 28. Dual-class virologic resistance was observed in 7 of 8 patients infected with genotype 1a. Addition of ribavirin delayed virologic failure, but still only 5 of 13 patients had levels of HCV RNA <25 IU/ml after 4 weeks. By contrast, quadruple therapy with tegobuvir, GS-9256, ribavirin and PEG-IFN-α2a was successful in all 14 patients treated. Several important conclusions can be drawn from this study that will be important for the future development of IFN-free therapies. First, combining two DAAs with rather low barriers to resistance and limited antiviral potency is not advised and will lead to resistance against both drugs within a few days of starting therapy. Secondly, ribavirin will still have an important role in IFN-free, all oral therapy of chronic hepatitis C—at least when weak DAAs are used. Thirdly, adding two DAAs to PEG-IFN-α–ribavirin therapy can be highly effective and might be an option for some difficult-to-treat patients.

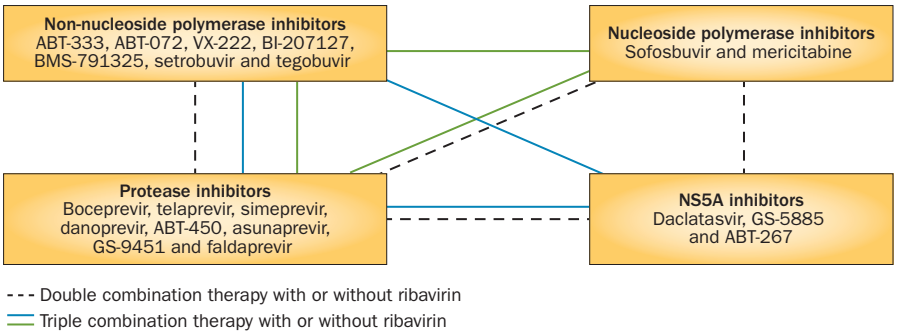

Figure 1 | IFN-free combination therapies of direct-acting antivirals against hepatitis C that are currently under investigation (including previously tested IFN-free combinations). The lines connect groups, from which preparations in an IFN-free combination therapy with or without ribavirin were used. Permission obtained from the German Liver Foundation (Deutsche Leberstiftung) © Maasoumy, B. & Wedemeyer, H. Interferon-freie Therapien der Hepatitis C: Wie ist der aktuelle Stand? *HepNet Journal* **6**, 6–8 (2012).

Until IFN-free therapy becomes reality, we have to use the present standard-of-care of PEG-IFN-α, ribavirin and HCV protease inhibitors. Currently, a key question in the treatment of chronic hepatitis C is whether all patients need protease inhibitors or if PEG-IFN-α–ribavirin alone is still sufficient in a subgroup of patients. In this latter context, it would be important to improve efficacy of the old standard-of-care, which might reduce the need for protease inhibitors. Japanese investigators explored whether raloxifene, a selective oestrogen receptor modulator, can increase response rates to standard PEG-IFN-α–ribavirin combination therapy.[7] The trial was based on the observation that postmenopausal women have very low response rates to IFN-based therapy. The investigators randomly assigned 123 women to receive PEG-IFN-α2a–ribavirin with or without raloxifene (60 mg per day). SVR rates were 34% in the control group, but 61% in women receiving raloxifene. On-treatment responses were higher and relapse rates were lower in patients who received raloxifene. The authors speculate that this marked increase in cure rates could be attributable to the antioxidant and lipid-peroxidation inhibiting activity of raloxifene. In addition, raloxifene might inhibit HCV infection at multiple steps of the HCV life cycle.[8] However, placebo-controlled trials are needed to confirm these interesting findings in other populations and in the context of triple therapy, or even in future IFN-free regimens.

A very important unresolved issue is whether therapy with telaprevir or boceprevir, which is very expensive, is cost-effective. In a mathematical model, Cammà and colleagues[9] addressed this question by comparing different approaches to the use of both protease inhibitors—either for all patients or only in patients not carrying the beneficial '*IL28B-CC*' genotype. Another scenario investigated was not to initiate boceprevir treatment in patients who achieved a rapid virologic response (RVR) after the 4-week PEG-IFN-α–ribavirin lead-in phase. In the applied model, triple therapy regimens increased survival by about 4 years with a fairly low cost. This data is very important for payers in many countries to justify coverage of treatment. Moreover, the study provides evidence supporting the use of cost-saving regimens that restrict treatment with a protease inhibitor to individuals who are likely to benefit most.

An enormous amount of additional new data on IFN-free treatments has been presented during the International Liver Congress organized by the European Association for the Study of the Liver in April 2012 and during the American

Key advances

- In 2012, the first cases of cure of chronic hepatitis C with an IFN-free therapy were reported,[4] although some IFN-free therapies were not successful[6]
- Until IFN-free regimens become available, clinicians must consider adverse events and the cost-effectiveness of current therapies[9] and adapt IFN regimens to increase sustained virologic response rates[7]

Association for the Study of Liver Diseases meeting in November 2012. Several studies confirmed that it is possible to cure HCV infection without IFN, even in patients with liver cirrhosis or in patients infected with HCV genotype 1a and other HCV genotypes. Of note, very high response rates can be expected. It is possible that more than three different regimens combining different DAA classes with or without ribavirin will reach the market by the end of 2014, which will not be the end of new developments in HCV therapy considering that at least 30–40 different novel compounds are currently being investigated in clinical trials. It is time to prepare to say goodbye to IFN!

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GLOMERULAR DISEASE IN 2012

More mechanistic insights, but translational progress is slow

Jeffrey B. Kopp

The year 2012 brought a continued harvest of new findings of relevance to glomerular biology and disease. Progress in glomerular disease has continued, although our understanding of disease processes continues to extend much further than our ability to intervene effectively.

Kopp, J. B. *Nat. Rev. Nephrol.* **9**, 67–68 (2013); published online 8 January 2013; doi:10.1038/nrneph.2012.288

2012 saw the publication of a number of studies relevant to the field of glomerular biology and disease. Two of the published advances related to glomerular diseases—one investigated the role of *APOL1* variation in glomerulosclerosis among African American people,[1] and another looked at the role of anti-phospholipase A_2 receptor (PLA_2R) antibodies in patients with membranous nephropathy.[2] In addition, three trials in diabetic nephropathy illustrated the difficulties of promising therapies succeeding in large-scale clinical trials.

In 2010, researchers showed that *APOL1* variation is associated with hypertensive end-stage renal disease (ESRD) in African American people.[3] In their 2012 study, Lipkowitz *et al.* studied earlier stages of arterionephrosclerosis (hypertensive nephrosclerosis) in participants of the African American Study of Kidney Disease (AASK).[1] They found that the presence of two *APOL1* risk alleles was associated with an increased risk of kidney disease in all case patients compared with controls (OR 2.57). The association between *APOL1* risk alleles and kidney disease was even stronger in those with baseline urinary protein-to-creatinine ratio >0.6 g/g (OR 6.29), and in those with a serum creatinine level >265 µmol/l during follow-up (OR 4.61).

During the randomized trial part of AASK, participants were randomly assigned to receive amlopidine, metoprolol, or ramipril. No interactions were seen between *APOL1* genotype and medication type or blood pressure response to medication, in terms of progressive loss of renal function. The results of these studies demonstrate that African American patients with hypertension and impaired glomerular filtration rate (GFR; the entry criteria for the AASK study) and two *APOL1* risk alleles represent individuals at high risk of progression to higher levels of proteinuria and advanced renal function impairment. The therapies employed in AASK did not seem to affect the relentless progression that characterizes these individuals, although without a control group we cannot be certain that a less-aggressive therapeutic approach would have been associated with even faster progression. New therapeutic approaches, going beyond control of blood pressure to current targets using existing agents, are needed for individuals with the *APOL1* risk genotype, but we as yet have little insight as to what those approaches might be.

Key advances

- *APOL1*-associated arterionephrosclerosis is aggressive, with a tendency to manifest increased proteinuria and to progress to more advanced kidney disease, despite aggressive therapeutic approaches[1]
- High levels of anti-PLA_2R antibodies suggest that spontaneous remission of membranous nephropathy may be unlikely; this information might contribute to the decision to institute immunosuppressive therapy[2]
- Aliskiren,[5] pyridorin[6] and sulodexide,[7] given in combination with conventional renin–angiotensin system antagonists, each failed to show benefit in patients with type 2 diabetes and overt nephropathy

In another important study in the field of glomerular disease in 2012, Hofstra *et al.* investigated levels of anti-PLA_2R antibodies in European patients with idiopathic membranous nephropathy and nephrotic-range proteinuria.[2] Antibodies to PLA_2R are present in up to 80% patients with idiopathic membranous nephropathy. Questions remain about the clinical meaning of anti-PLA_2R antibody titres, which are assessed either using indirect immunofluorescence staining using HEK 293 cells stably transfected with PLA_2R, or by ELISA testing.

Hofstra and colleagues studied samples from 117 patients with membranous nephropathy from Paris (France), Nijmegen (The Netherlands), and Manchester (UK), representing the largest series published to date.[2] Overall, 72–74% of patients had anti-PLA_2R antibodies by one or both assays. A modest correlation was found between anti-PLA_2R titres measured by the two methods ($r = 0.679$). Previous work had suggested that pathogenic anti-PLA_2R antibodies tend to be of the IgG4 subclass,[4] and Hofstra *et al.*'s study had similar findings, with 69% of patients having antibodies of the IgG4 subclass. A robust correlation was shown between IgG4 ELISA titre and immunofluorescence staining ($r = 0.880$). Antibody titre measured by ELISA correlated positively with baseline proteinuria but the relationship was weak ($r = 0.259$, $P = 0.02$). In the Dutch subcohort, the relationship tended to be stronger, and was further strengthened when serum IgG titre was adjusted for urinary IgG losses. No statistically significant differences were found in responses to immunosuppressive therapy or in the rate of spontaneous remissions between individuals with and without anti-PLA_2Rs. The most relevant finding from a clinical standpoint was that when anti-PLA_2R-seropositive subjects were grouped into tertiles based on ELISA titre, those in the highest tertile had a lower rate

of spontaneous remission (4%) than those in the middle tertile (31%) and those in the lowest tertile (38%) ($P<0.01$). The picture that emerges is that ~75% patients with idiopathic membranous nephropathy have anti-PLA_2R antibodies, and that those with high titres are less likely to enter spontaneous remission, a finding that might have clinical predictive value.

The year was a disappointing one for studies testing novel treatment approaches in type 2 diabetic nephropathy. Studies employing aliskiren,[5] pyridorin[6] and sulodexide[7] (combined with an angiotensin-converting-enzyme [ACE] inhibitor or an angiotensin-receptor blocker [ARB]) each failed to show therapeutic beneficial effects in well-designed, well-executed, and suitably powered randomized controlled trials involving subjects with macroproteinuria and/or impaired GFR. Each molecular entity had attractive features, with two of the agents targeting pathways known to be active in diabetic nephropathy. Aliskiren inhibits renin activity, and ACE inhibitors and ARBs are known to be effective therapies in diabetic nephropathy. Pyridorin, a derivative of vitamin B6 (pyridoxal phosphate), inhibits the formation of advanced glycation end-products and also scavenges reactive carbonyl species, which would inhibit lipid oxidation products. Sulodexide is a mixture of four glycosaminoglycans derived from porcine lung and liver; beneficial effects had been shown in a pilot clinical trial in patients with diabetic nephropathy although the mode of action remains uncertain.[8]

The study sizes for the 2012 studies of novel agents in diabetic nephropathy were as follows: 8,561 patients in the aliskiren study, 317 patients in the pyridorin study and 1,248 patients in the sulodexide study. All patients were receiving background therapy with an ACE inhibitor or an ARB. Primary end points included measures of progression of chronic kidney disease. For the aliskiren study, the renal (secondary) end point was a composite of: ESRD, death attributable to kidney failure, need for renal replacement therapy with no dialysis or transplantation available or started; or a doubling of the baseline value of serum creatinine level with a value exceeding the upper limit of the normal range. After a mean of 2.7 years, the secondary renal end point had occurred in similar numbers of patients in the aliskiren and placebo groups (HR 1.03 in aliskiren group, 95% CI 0.87–1.23; $P=0.74$) and the trial was stopped early after an interim efficacy analysis.[5] In the pyridorin study, the primary end point—change in serum creatinine concentration at 1 year—showed no differences for either the 150 mg or the 300 mg dose of pyridorin (each given twice daily) compared with placebo ($P=0.48$ and $P=0.95$, respectively).[6] For the sulodexide study, the primary end point was time to doubling of baseline serum creatinine level, development of ESRD, or a serum creatinine level ≥530 μmol/l. After a mean duration of 11 months, the number of primary end point events was not significantly different in the sulodexide treatment group compared with the placebo group (HR 0.85, 95% CI 0.50–1.44; $P=0.54$).[7] A companion study by the same collaborative group also published in 2012 showed that sulodexide did not reduce albuminuria in patients with type 2 diabetes and microalbuminuria either.[9]

> “...a translational gap still exists...”

So why are the results from these studies important, despite the fact that they each failed to reject the null hypothesis? Each therapeutic agent had been the subject of prior preclinical investigations that showed promise and these well-designed and well-executed studies did not find even a trend towards a beneficial effect. Are the doors now closed to these agents? For pyridorin, the authors state that an exploratory analysis suggested a beneficial effect in those with preserved GFR. For sulodexide, although the study was terminated early for reasons that were not stated but may have been due to considerations of futility, the study had power to detect a 20% reduction in proteinuria, which was not seen. Given the negative results from these high-quality studies, it would seem at present unlikely that pyridorin or sulodexide will be studied further as single agents added onto background ACE inhibitor or ARB therapy for diabetic nephropathy but they might conceivably be tested as part of multidrug combination therapies. With regard to combination renin–angiotensin system (RAS) antagonist therapy, the combination of aliskiren plus an ACE inhibitor or an ARB was ineffective; in addition, the 2008 ONTARGET study of individuals with advanced atherosclerotic disease showed that the combination of an ACE inhibitor plus an ARB was equivalent to either agent alone in terms of cardiovascular outcomes, but was associated with an increased risk of adverse renal outcomes.[10] So although combination RAS antagonist therapy is widely used for reducing proteinuria in nephrotic diseases, no convincing evidence yet exists that such combinations are beneficial in diabetic nephropathy and they may be unwise in patients who have glomerular disease and advanced atherosclerotic disease.

In conclusion, considering research into glomerular disease in 2012, the advice to ‘mind the gap’ remains operative—a translational gap still exists between our understanding of disease mechanisms and our ability to devise effective therapy. We hope and can perhaps expect that we will do better in the future.

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CARDIOVASCULAR DISEASE IN CKD IN 2012

Moving forward, slowly but surely

Pranav S. Garimella and Mark J. Sarnak

During 2012, an observational study confirmed the high risk of cardiovascular disease ascribed to chronic kidney disease (CKD) and again raised the question of whether CKD should be considered a cardiovascular disease risk equivalent. Several other studies evaluated methods to mitigate cardiovascular risk in CKD. The results of these studies have advanced the field but have also raised more questions.

Garimella, P. S. & Sarnak, M. J. **9**, 69–70 (2013); published online 8 January 2013;
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Key advances

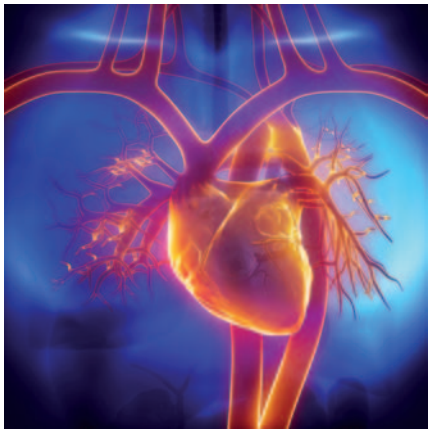
- Observational data support the hypothesis that individuals with kidney disease, especially those with both reduced glomerular filtration and proteinuria, are at extremely high risk of adverse cardiac events[2]
- A low-sodium diet in individuals treated with an angiotensin-receptor blocker may be synergistic in treating hypertension, preventing a decline in kidney function and reducing the risk of cardiovascular events[3]
- Warfarin may be associated with a reduced risk of stroke in patients with chronic kidney disease (CKD) and atrial fibrillation, but more studies are required in this patient population[6]
- Activated vitamin D[8] and calcimimetic agents[9] have not conclusively been shown to reduce the risk of cardiovascular disease in CKD

Nearly 10 years ago, the American Heart Association (AHA) recommended that people with chronic kidney disease (CKD) should be considered in the highest-risk group for the prevention, detection, and treatment of cardiovascular risk factors.[1] Since the publication of this statement, there has been debate in the literature as to whether CKD is a cardiovascular disease (CVD) risk equivalent. A 2012 observational study of 1,268,029 individuals added to this literature.[2] Among those without previous myocardial infarction (MI), the unadjusted rate of MI was higher in people with CKD (without diabetes) than in those with diabetes (without CKD) (6.9 per 1,000 person-years versus 5.4 per 1,000 person-years [95% CI 6.6–7.2 versus 5.2–5.7]).[2] When CKD was defined by an estimated glomerular filtration rate (eGFR) of <45 ml/min/1.73 m^2 and proteinuria (rather than the primary definition of eGFR <60 ml/min/1.73 m^2), the risk of incident MI was nearly twice as high in those with CKD than in those with diabetes (12.4 per 1,000 person-years versus 6.6 per 1,000 person-years [95% CI 9.7–15.9 versus 6.4–6.9]). All-cause mortality was highest among people with diabetes and CKD, followed by those with CKD alone, then those with previous MI and those with diabetes alone. Although constrained by the limitations of observational studies, the results of this study again highlight the increased CVD burden associated with CKD, especially among those with both reduced eGFR and proteinuria.

> “Despite many negative results ... there does seem to be light at the end of the tunnel”

Blood pressure control remains an important modifiable risk factor for both slowing the progression of CKD and the development of CVD. A *post hoc* analysis including 1,117 participants from the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL) and Irbesartan Diabetic Nephropathy Trial (IDNT) trials, evaluated the effect of dietary sodium intake (assessed by 24 h urine sodium excretion, a surrogate for salt intake) on the efficacy of angiotensin-receptor blockers (ARBs) in preventing clinically important CKD and CVD end points compared with non-ARB therapy.[3] Among those randomized to ARB therapy, the investigators noted that absolute reductions in 24 h urinary albumin-to-creatinine ratio (ACR; measured in mmol/g) and systolic blood pressure were greatest in those with the lowest baseline urinary sodium-to-creatinine ratio. A low sodium-to-creatinine ratio was not associated with a significant decrease in renal or CVD events in non-ARB-treated subjects; however, among those treated with ARBs, individuals in the lowest tertile of sodium-to-creatinine ratio experienced significantly fewer CVD events than those in the highest tertile of sodium-to-creatinine ratio (hazard ratio 0.57 versus 1.37 [95% CI 0.39–0.84 versus 0.96–1.96]). Importantly, ARB therapy was not associated with a significant reduction in systolic blood pressure, renal or CVD events among those in the highest tertiles of sodium-to-creatinine ratio. These results are consistent with those from a *post hoc* analysis of the Ramipril Efficacy in Nephropathy trials (REIN I and II), which demonstrated that low salt intake was associated with a lower risk of end-stage renal disease (ESRD) than was a higher salt intake in nondiabetic patients with proteinuria being treated with angiotensin-converting-enzyme (ACE) inhibitors.[4] All of these findings should be considered in the context of other recent observational data (with their attendant limitations) in the general population showing a ‘J’-shaped association between salt excretion and cardiovascular risk.[5]

Whether warfarin is associated with a reduced risk of stroke among patients with CKD, especially those on dialysis with non-valvular atrial fibrillation, remains controversial. A 2012 study of 132,372 people with atrial fibrillation included in Danish national registries noted that although individuals with CKD had an increased risk of stroke compared with those without kidney disease, this risk was significantly attenuated by the use of warfarin (hazard ratio 0.76; 95% CI 0.64–0.91).[6] The reduction in stroke risk seen with warfarin was even stronger when the analysis was limited to patients on dialysis (hazard ratio 0.44 [95% CI 0.26–0.74]). Bleeding complications associated with warfarin were more frequent in individuals with CKD than in those without CKD (hazard ratio 1.33 [95% CI 1.16–1.53]). These results are in contrast to those from an earlier observational study of patients on dialysis, which indicated that those treated with warfarin are in fact at an increased risk of stroke.[7] The older age of the dialysis patients (mean 73 years versus 67 years) and the high number of patients on warfarin without international normalized ratio (INR) monitoring (27%) in the earlier study, together with differences in the ascertainment of stroke outcomes, may account for some of the differences. A high-quality randomized trial of warfarin in patients on dialysis with atrial fibrillation would help answer the question of whether warfarin has a beneficial effect on the risk of



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stroke. Given the known benefit of warfarin in the general population, however, conducting such a trial and deciding in which patient group sufficient clinical equipoise exists to perform a randomized trial would be challenging. Until further data are available, we suggest that in CKD patients with an elevated risk of stroke according to $CHADS_2$ score, treatment with warfarin should be considered on an individual basis and only given with close INR monitoring and after weighing the increased risk of bleeding.

2012 also saw the publication of results from two clinical trials targeting abnormalities in mineral metabolism in patients with CKD. Thadhani and colleagues evaluated the effect of treatment with paracalcitriol versus placebo in 227 individuals with a serum parathyroid hormone level between 50 pg/ml and 300 pg/ml, an eGFR within the range 15–60 ml/min/1.73 m^2 and mild to moderate left ventricular hypertrophy (LVH).[8] At 48 weeks, no differences were seen between the two groups in left ventricular mass index or any of the 10 other assessed parameters of cardiac structure or function. Although earlier studies in rat models had demonstrated that vitamin D supplementation was associated with a decrease in LVH, current clinical data do not support supplementation with active vitamin D for altering cardiac structure in patients with CKD.

In the EVOLVE (Effect of Cinacalcet on Cardiovascular Disease in Patients Undergoing Dialysis) trial, 3,883 dialysis patients with secondary hyperparathyroidism were randomly assigned to receive cinacalcet or placebo. In the non-adjusted intention-to-treat analysis, cinacalcet was not associated with a reduction in the composite primary outcome of death, MI, hospitalization for unstable angina, heart failure, or a peripheral vascular event (hazard ratio 0.93 [95% CI 0.85–1.02]).[9] Interestingly, after adjustment for baseline characteristics, a significant reduction in the relative hazard of the composite end point was noted in the cinacalcet group (hazard ratio 0.88 [95% CI 0.79–0.97]); however, such an adjustment was not pre-specified in the protocol for the primary outcome. The trial may also have been biased towards the null hypothesis given that commercial cinacalcet was used in nearly 20% of the placebo group. Therefore, although some aspects of the trial are suggestive of a beneficial effect of cinacalcet, the primary outcome was negative and the conclusions are nondefinitive.

Despite many negative results from recent randomized controlled trials in patients with CKD, there does seem to be light at the end of the tunnel. In fact, mortality adjusted for age, gender, race, and comorbid conditions among prevalent ESRD patients has fallen 28.4% since 1995.[10] Although the exact reasons for this finding remain unclear, it is encouraging. Several simultaneous interventions are probably required for a reduction in risk to be demonstrated, given the high burden of different forms of CVD and multiple traditional and nontraditional CVD risk factors in this patient population.

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RENAL VASCULITIS IN 2012

Reclassification and the introduction of biologicals

Cees G. M. Kallenberg

2012 saw the classification of the systemic vasculitides revised. Genetic studies showed that granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA) are different diseases with aberrant immune responses to different autoantigens. B-cell depletion with rituximab also acquired a primary role in the treatment of GPA and MPA, as well as in cryoglobulinaemic vasculitis.

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The kidney is frequently involved in systemic vasculitis and renal involvement is a major determinant of treatment choice and outcome. The definition of vasculitides is relevant, not only for the inclusion of homogeneous populations in clinical trials, but also for applying results from these trials to individual patients. In 2012, a new nomenclature system for the vasculitides was published with definitions for

each form of vasculitis.[1] The 2012 Revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides aimed to define vasculitides based on the size of the vessels involved, immunohistopathology, localization, and probable aetiology. On the basis of this framework, diagnostic criteria are now being developed in order to better classify individual patients.

Ideally, classification of any disease is based on its aetiopathogenesis. A major step forward for the antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides was the discovery that the genetic background of granulomatosis with polyangiitis (GPA; formerly known as Wegener granulomatosis) is different to that of microscopic polyangiitis (MPA).[2] The strongest genetic associations, however were with the autoantigen specificity of ANCA rather than with the clinical syndrome. Antiproteinase 3 antibodies—markers of GPA—were associated with HLA-DP as well as with genes encoding proteinase 3 and its inhibitor α1-antitrypsin, whereas antimyeloperoxidase antibodies—markers of MPA—were associated with HLA-DQ.[2] These data not only underscore the fact that GPA and MPA are distinct diseases but also that the immune responses to proteinase 3 and myeloperoxidase underlie the immunopathogenesis of GPA and MPA, respectively.

In 1979, a major breakthrough in the treatment of severe systemic vasculitis was the introduction of oral cyclophosphamide; however, the adverse effects associated with long-term oral cyclophosphamide treatment are considerable. The European Vasculitis Study Group (EUVAS) have attempted, through a number of controlled trials, to test whether eliminating or reducing the cumulative dose of cyclophosphamide is feasible. In one of these trials, azathioprine was shown to be equivalent to oral cyclophosphamide for maintenance of remission.[3] Another study found that pulse cyclophosphamide was as effective as daily oral cyclophosphamide for induction of remission, with an almost 50% reduction of the cumulative dose of cyclophosphamide.[4] There was, however, a suggestion that pulse cyclophosphamide would lead to more relapses and a worse outcome in the long term. In 2012, the long-term outcomes of 148 patients from this study were published (median follow-up 4.3 years).[5] The findings showed a higher relapse rate in patients treated with pulse cyclophosphamide than in those treated with daily oral cyclophosphamide (39.5% versus 20.8%, $P = 0.029$), but mortality, long-term morbidity and renal function did not differ between the groups. Thus, given the short-term and long-term adverse effects of cyclophosphamide, pulse cyclophosphamide seems to be preferable over daily oral cyclophosphamide for induction of remission. Long-term follow-up of a trial in which methotrexate was compared with daily oral cyclophosphamide, however, showed that induction treatment with methotrexate led to less effective disease control than did induction with cyclophosphamide.[6]

Key advances

- Granulomatosis with polyangiitis and microscopic polyangiitis have different genetic associations, pointing towards a primary pathogenic role for their associated autoantibodies[2]
- Although associated with more frequent relapses, induction treatment with intravenous cyclophosphamide for antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) has comparable long-term outcomes to daily oral cyclophosphamide[5]
- B-cell-depleting therapy with rituximab seems effective in patients with refractory AAV and can probably also be used for maintenance treatment[8]
- Rituximab seems more effective than conventional therapy for both noninfectious and hepatitis C virus-associated cryoglobulinaemic vasculitis[9,10]

> “These findings might allow us to abandon cyclophosphamide...”

In 2010, a large controlled trial of 197 patients with severe ANCA-associated vasculitis, 132 of whom had renal involvement, showed that an induction regimen of rituximab without cyclophosphamide was as effective as a regimen based on oral cyclophosphamide for induction of remission. In patients with relapsing disease, rituximab was even more effective than cyclophosphamide.[7] Although long-term follow-up data are still to be published, preliminary data suggest that rituximab could replace cyclophosphamide in the treatment of ANCA-associated vasculitis. This suggestion is strengthened by a study from the Mayo Clinic, USA, describing the outcomes of 53 patients with chronic refractory GPA who were treated with at least two courses of rituximab.[8] 32 patients experienced relapse; all 32 relapses occurred after reconstitution of B cells and all were accompanied or preceded by an increase in ANCA levels, except in one patient who was ANCA negative. Relapses were successfully treated with rituximab and all patients achieved complete remission. Moreover, pre-emptive treatment with rituximab, given after reconstitution of B cells and increase in ANCA levels in most cases (130 of 148 courses), led to maintenance of remission. These promising data suggest that intermittent treatment with rituximab can maintain remission in patients with relapsing ANCA-associated vasculitis. A prospective study comparing azathioprine with rituximab for maintenance of remission (RITAZAREM) is underway.

Rituximab has also been used in patients with cryoglobulinaemic vasculitis. Terrier *et al.*[9] retrospectively analysed data from 242 patients with noninfectious cryoglobulinaemic vasculitis, 35% of whom had glomerulonephritis in their clinical presentation. The disease was essential in 48% of patients, associated with a connective tissue disease in 30% and associated with B-cell non-Hodgkin lymphoma in 22%. After correction for time-dependent confounders, rituximab with corticosteroids was more efficacious than corticosteroids alone or alkylating agents with corticosteroids for clinical, renal, and immunological responses and for corticosteroid-sparing effects. However, the rituximab plus corticosteroids regimen was associated with more severe infections than the other regimens. De Vita *et al.*[10] performed a prospective, randomized controlled trial of 59 patients with cryoglobulinaemic vasculitis, the majority of whom ($n = 53$) were positive for hepatitis C virus (HCV) infection. Of the 53 HCV-positive patients, antiviral therapy had been ineffective or poorly tolerated in 25 patients and was considered contraindicated in 28. Patients were randomly allocated to receive either rituximab with corticosteroids (two infusions of 1 g with tapering of corticosteroids and a second course of rituximab at relapse) or conventional treatment (consisting of corticosteroids only, cyclophosphamide or azathioprine with or without steroids, or plasma exchange with or without steroids). The primary end point—the proportion of patients still on their initial therapy at 12 months—was far higher in the rituximab group than in the conventional-treatment group (64.3% versus 3.5%; $P<0.0001$). Rituximab seemed superior for all three target organ manifestations—skin ulcers, glomerulonephritis, and peripheral neuropathy. Moreover, response to rituximab was seen in 14 out of

23 patients who failed conventional therapy (60.9%). Although this study was relatively small, the data strongly suggest that rituximab with a tapering dose of steroids is an attractive treatment for patients with HCV-associated cryoglobulinaemic vasculitis who fail or are not eligible for antiviral treatment.

In conclusion, data from 2012 demonstrate the potential of rituximab for the induction and maintenance treatment of various forms of systemic vasculitis with renal involvement, in particular ANCA-associated vasculitis and cryoglobulinaemic vasculitis. These findings might allow us to abandon cyclophosphamide in the treatment of these life-threatening conditions.

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Competing interests
The author declares no competing interests.

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ACUTE KIDNEY INJURY IN 2012

Type of resuscitation fluid—it does matter!

Antoine G. Schneider and Rinaldo Bellomo

2012 saw the publication of four important trials investigating the choice of fluid therapy in patients suffering from critical illness or undergoing major surgery. These studies pave the way for more evidence-based administration of fluid in such patients.

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Administration of intravenous fluids for volume expansion is very common in critically ill patients, particularly in the early stages of sepsis or during the perioperative period. This procedure is often regarded as routine and delegated to junior doctors. However, high-quality evidence on the most appropriate type of fluid to be administered in such an instance is limited and no consensus exists. Colloids, which contain large insoluble molecules such as starch or gelatine, are thought to enable faster and more effective intravascular expansion and result in less oedema than crystalloids. However, colloids are associated with higher cost and those based on starch may accumulate in the tissue and could be associated with acute kidney injury.[1] Despite these issues, current guidelines for early sepsis management[2] still recommend colloids and crystalloids equally. Thus colloids—particularly those based on starch—continue to be widely used throughout the world.[3]

2012 saw the publication of two major clinical trials that compared the administration of starch solutions with the administration of crystalloids in critically ill patients. The first of these trials—the Scandinavian Starch for Severe Sepsis/Septic Shock (6S) trial—was a well-conducted, multicentre, double-blind, parallel-group trial in which 804 critically ill patients with severe sepsis were randomly assigned to fluid resuscitation with 6% hydroxyethyl starch (HES) 130/0.4 or Ringer's acetate.[4] The key findings of the trial were striking. First, contrary to most common assumptions, patients allocated to receive HES did not require a decreased volume of fluids to achieve resuscitation targets compared with patients assigned to Ringer's acetate. In addition, patients assigned to HES had an increased in-hospital mortality rate at 90 days (relative risk [RR] 1.17 versus Ringer's acetate group; $P = 0.03$) as well as an increased use of renal replacement therapy (RR 1.35; $P = 0.04$). However, because patients in this study were a unique cohort of patients with severe sepsis or septic shock, it was unclear whether these findings could be translated to other critically ill patients.

Later in 2012, the results of a larger-scale trial, the Crystalloids versus Hydroxyethyl Starch Trial (CHEST), were reported.[5] This trial, the largest double-blind, randomized controlled trial in critically ill patients to date, compared use of HES 130/0.4 with use of 0.9% saline for fluid resuscitation in 7,000 critically ill patients. In CHEST, overall in-hospital mortality (17.5%) was lower than expected and no difference was detected between the two groups in 90-day mortality. However, similar to the findings of the 6S trial, more patients allocated to receive HES required renal replacement therapy (RR 1.21; $P = 0.04$). In addition, HES-treated patients experienced a higher

Key advances

- Use of 6% hydroxyethyl starch (HES) 130/0.4 for fluid resuscitation is associated with increased in-hospital mortality and an increased need for renal replacement therapy compared with Ringer's acetate in patients with severe sepsis[4]
- Use of 6% HES 130/0.4 is associated with an increased need for renal replacement therapy in a general intensive care population[5]
- Liberal administration of chloride-rich solutions might be associated with an elevation in mean serum creatinine level, an increase in the incidence of acute kidney injury and an increase in the need for renal replacement therapy[9]
- In surgical patients, normal saline might be associated with a significant increase in the need for renal replacement therapy compared with use of Plasma-Lyte®[10]

rate of adverse events (5.3% versus 2.8%, $P = 0.001$). *Post hoc* analysis showed that, during the first 7 days after randomization, serum creatinine levels were increased and urine output was decreased in the HES group, as compared with the saline group ($P = 0.004$ and $P = 0.003$, respectively).

Taken together and in the context of previous observations with other starches of high molecular weight, these results confirm that HES administration does not carry any significant advantage over administration of crystalloids for fluid resuscitation. On the contrary, they indicate that HES use might be associated with harm. As HES fluids have higher associated costs than crystalloids, it seems reasonable to conclude that such fluids should not be used in critically ill patients.

If, in the light of these findings, starch can now be considered a nephrotoxin, two other studies published in 2012 actually suggested that similar concerns might apply, although perhaps to a lesser extent, to 0.9% saline—the most commonly used intravenous solution in the world.[6] Multiple studies have shown that 0.9% saline is associated with the development of metabolic acidosis and that its high chloride content may affect kidney function.[7] In particular, it is thought that the administration of large quantities of chloride anion could lead to a reduction in glomerular filtration rate via a tubuloglomerular feedback mechanism and afferent arteriolar vasoconstriction.[8]

One of the 2012 saline trials examined the association between a chloride-liberal or a chloride-restrictive fluid administration strategy and acute kidney injury in critically ill patients.[9] In this single-centre study, any use of chloride-rich intravenous fluids (0.9% saline, 4% succinylated gelatin solution and 4% albumin in sodium chloride) was restricted to being used only after prescription by the attending specialist approval for specific conditions for a period of 6 months (chloride-restrictive period). The outcomes of the 773 patients admitted during this period were compared with those of the 760 patients admitted to the same unit during a previous 6-month period (chloride-liberal period). The intervention resulted in an average decrease in the amount of chloride anion administered from 694 mmol to 496 mmol per patient. Overall, no differences in mortality or length of stay in hospital or the intensive care unit were observed between the two periods. However, patients admitted during the chloride-restrictive period showed a smaller increase in mean serum creatinine level, a lower incidence of severe acute kidney injury (RIFLE class I or F) and less use of renal replacement therapy compared with those admitted during the chloride-liberal period. These differences persisted after adjustment for covariates.

These findings are consistent with those obtained in another 2012 study—a large retrospective cohort analysis that used data from an automated hospital claims database in the USA.[10] In this study, Shaw *et al.* analysed data from more than 30,000 patients who exclusively received either 0.9% saline or Plasma-Lyte® (Baxter International, Deerfield, IL, USA), a balanced crystalloid solution, on the day of a major non-traumatic general surgical procedure. After adjustment for baseline imbalances performed with several statistical models including a propensity score, no difference was observed between the two groups in terms of in-hospital mortality (except in the emergency surgery subgroup [odds ratio 0.51; 95% CI 0.28–0.95 in favour of Plasma-Lyte®]) or major complications. However, patients who received 0.9% saline required dialysis five times more often than those who received Plasma-Lyte®. In addition, saline-treated patients had a higher incidence of postoperative infections and received more tests (arterial blood gases and lactic acid levels) and interventions (buffer administration, fluids and blood) than those who received Plasma-Lyte®.

Taken together, the results from these studies suggest a possible degree of 'nephrotoxicity' of chloride-containing solutions when administered on a large scale. However, due to the before-and-after or observational design of the studies, care should be taken in the interpretation of the results. Further studies are required to confirm or refute these provocative findings. Nonetheless, given the absence of evidence of harm, clinicians might prefer to use balanced solutions rather than saline when large amounts of crystalloids are given.

Over the past 12 months, good-quality evidence has emerged that should enable clinicians to make better decisions when selecting which fluid to administer to a critically ill patient or indeed a patient having major surgery. Above all, the aforementioned studies suggest that intravenous fluids should be regarded as drugs, that the choice of fluid type does matter and that the decision to administer a particular kind of fluid should be considered carefully.

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Competing interests
The authors declare no competing interests.

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DIALYSIS IN 2012

Could longer and more frequent haemodialysis improve outcomes?

Rajnish Mehrotra and Jonathan Himmelfarb

Patients with end-stage renal disease typically receive three 3–4 h haemodialysis sessions per week. Although available data from well-powered randomized trials are limited, studies published in 2012 provided new evidence that haemodialysis regimens with longer treatment times and/or a higher frequency of sessions might reduce the high morbidity and mortality of patients on maintenance dialysis.

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Box 1 | Haemodialysis treatment regimens

In-centre haemodialysis
- Conventional regimen (three sessions per week, 3–4 h per session)
- Diurnal long-duration (three or more sessions per week, ≥5.5 h per session)
- Nocturnal long-duration (three or more sessions per week, ≥5.5 h per session)

Home haemodialysis
- Diurnal, thrice-weekly using conventional machines (3–4 h per session)
- Frequent using conventional or low-flow machines (more than three sessions per week, 2–4 h per session)
- Frequent long-duration nocturnal (three or more sessions per week, ≥5.5 h per session)

The majority of patients with end-stage renal disease (ESRD) receive in-centre haemodialysis three times per week in sessions lasting 3–4 h. Logistical factors, including patient and provider convenience and economic considerations, have perpetuated the use of this conventional haemodialysis regimen. However, in 2012 a reappraisal of alternative approaches to delivering haemodialysis (Box 1) occurred in response to longstanding concerns that the high morbidity and mortality of patients with ESRD might be, at least in part, directly attributable to the inherently limited solute clearance and volume removal that is achieved with conventional dialysis prescriptions.

Theoretical advantages of longer haemodialysis sessions include slower and potentially safer ultrafiltration, reduced intradialytic hypotension, and more effective clearance of solutes that have a higher molecular weight than urea (such as β2-microglobulin) or that equilibrate slowly across different body compartments (such as phosphorus). However, several developments in the 1980s and 1990s led to a reduction in emphasis on the importance of haemodialysis treatment time. Firstly, in 1981 a randomized controlled clinical trial with 151 participants, the National Cooperative Dialysis Study, showed no statistically significant effect of dialysis session length (4.5–5.0 h versus 2.5–3.5 h) on patient morbidity or mortality ($P = 0.06$).[1] Secondly, there was a shift towards the sole use of Kt/V_{urea} (that is, the clearance of urea multiplied by time and normalized for distribution volume) for quantification of dialysis dose. This approach was bolstered by observational studies that demonstrated an association between Kt/V_{urea} and patient survival. Finally, the introduction of high-flux dialysers allowed 'adequate' Kt/V_{urea} targets to be achieved in a comparatively short treatment time.

Nonetheless, some interest in longer haemodialysis treatment times has persisted, mainly as a result of reports of superior patient outcomes with extended treatment times in Tassin, France (~24 h per week),[2] and subsequently with nocturnal home haemodialysis in Toronto, Canada (~40 h per week).[3] Although these studies were not controlled, the findings led to an increase in the use of alternative haemodialysis treatment regimens with longer treatment times, particularly thrice-weekly nocturnal in-centre haemodialysis in the USA. An observational study published in 2012 showed that the mortality risk of 746 patients treated with thrice-weekly nocturnal in-centre haemodialysis (mean treatment time per session of 471 min) was 25% lower than that of 2,062 patients who received conventional in-centre haemodialysis.[4] These data strengthen the argument for longer haemodialysis treatment duration even in the absence of a higher frequency of dialysis sessions. A pragmatic clinical trial, sponsored by the NIH, is being planned to investigate this issue.

> “...in 2012 a reappraisal of alternative approaches to delivering haemodialysis occurred...”

In recent years the effect of increasing the frequency of haemodialysis on patient outcomes has also garnered increased attention. An increased mortality risk during the 72 h weekend treatment interval in patients receiving conventional thrice-weekly haemodialysis has been recognized for more than a decade, and was confirmed in a large observational study published in 2011.[5] The potential benefits of more frequent haemodialysis treatments, together with the development of easy-to-use dialysis machines, have led to an increase in the utilization of frequent home haemodialysis. In the USA, this growth has been driven by patients using the NxStage System One haemodialysis system. Notwithstanding the benefits of an increased frequency of treatment, given the lower clearances obtained with this system it cannot be considered equivalent to the regimens that were evaluated in previous observational studies or in a recent clinical trial.[6] An observational study published in 2012 showed that patients with ESRD who received daily home haemodialysis using Nxstage System One ($n = 1,873$) had 13% lower all-cause mortality and 8% lower cardiovascular mortality than a matched cohort of patients from the US Renal Data System database who received conventional thrice weekly in-centre haemodialysis ($n = 9,365$).[7] These findings raise the possibility that more frequent haemodialysis regimens might improve volume control with safer ultrafiltration during treatment even without increased solute clearance rates.

The effect of more frequent dialysis, in some cases combined with extended treatment times, has been investigated in a number of randomized controlled trials.[6,8,9] In 2010, a randomized controlled trial showed a beneficial effect of frequent, short-duration (mean of 5.2 sessions of 154 ± 25 min per week) in-centre haemodialysis compared with a conventional regimen (mean of 2.9 sessions of 213 ± 28 min per week).[6] The shorter, more frequent treatment regimen was associated

with statistically significant improvements in the coprimary composite outcomes of death or change in left ventricular mass, and death or 12-month change in a self-reported physical health composite score.[6] These improvements might have been mediated by factors including safer ultrafiltration, improvements in volume status, a reduction in blood pressure or lowering of serum phosphorus levels. The trial was not powered to demonstrate a reduction in risk of death and there were no apparent improvements in hospitalization rates, nutritional status or objective physical performance in the patients who received more frequent haemodialysis. Moreover, the more frequent haemodialysis regimen was associated with an increased frequency of vascular access interventions. To date, economic and logistical considerations have precluded health care providers from offering more frequent in-centre haemodialysis to patients with ESRD.

If increases in both the duration and frequency of haemodialysis sessions could be beneficial, the question that follows is whether a treatment regimen that combines longer session durations with an increased treatment frequency would have even greater benefits. In 2012, the results of the first (and to date only) observational study to have examined such a regimen were published. In this retrospective study, data from 338 patients who received an intensive haemodialysis regimen (mean of 4.8 sessions of mean 441 min per week) were compared with those from 1,388 matched patients who received conventional haemodialysis (mean of three sessions of mean 236 min per week).[10] The patients who received the more intensive haemodialysis regimen had a 45% lower risk of death than those who received conventional thrice-weekly haemodialysis.[10]

Two previous randomized controlled clinical trials compared the effect of frequent (5–6 times per week) nocturnal home haemodialysis to conventional in-centre or home dialysis regimens with somewhat mixed results.[8,9] Both studies showed beneficial effects of the nocturnal home haemodialysis regimen on blood pressure lowering and serum phosphorous levels. However, one trial showed that nocturnal haemodialysis improved left ventricular mass,[8] whereas the other did not demonstrate any beneficial effect of nocturnal haemodialysis on this outcome.[9] Unfortunately both trials were underpowered for ascertainment of many important clinical outcomes, such as hospitalization or mortality.

Key advances

- The limited solute clearance and volume removal associated with conventional haemodialysis regimens might contribute to the high morbidity and mortality of patients with end-stage renal disease
- Observational studies published in 2012 demonstrated that longer haemodialysis treatment duration, delivered either thrice-weekly[4] or more frequently,[10] is associated with lower patient mortality than conventional haemodialysis regimens
- In 2012, an observational study showed that a higher frequency of haemodialysis using a system that delivered lower solute clearances reduced patient mortality compared with a conventional haemodialysis regimen[7]
- Continued expansion of haemodialysis treatment regimens with longer treatment times and/or higher frequency is likely although the new observations are preliminary and need to be validated in clinical trials

In conclusion, we have highlighted three observational studies published in 2012 that provide new evidence of beneficial effects of longer treatment time and/or greater frequency of haemodialysis on patient outcomes.[4,7,10] However, it is important to recognize the limitations of these studies. Given their observational nature, caution should be exercised when attributing the beneficial effects of longer and/or more frequent haemodialysis regimens to the regimen itself rather than to the characteristics of the patients selected for treatment using these alternative approaches. Moreover, the studies controlled only for baseline differences in patient characteristics and not for changes in these characteristics over time. Despite these limitations, concerns about the health risk imposed by conventional haemodialysis regimens is likely to continue to drive an expansion in use of longer haemodialysis session times and greater haemodialysis frequency, both at home and in-centre. In the meantime, there is a continued need to validate the preliminary findings using well-powered, randomized controlled clinical trials and sophisticated epidemiologic methods.

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Competing interests
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MOTOR NEURON DISEASE IN 2012

Novel causal genes and disease modifiers

Rosa Rademakers and Marka van Blitterswijk

In 2012, researchers published extensively on the genetic and clinicopathological characterization of patients with the newly discovered *C9ORF72* repeat expansions, which cause amyotrophic lateral sclerosis (ALS) and frontotemporal dementia. Novel ALS-linked genes and genetic modifiers were identified through screening in animal models and patients.

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Towards the end of 2011, two independent groups identified the long-sought-after cause of chromosome 9p-linked amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD).[1,2] They revealed a gene defect in chromosome 9 open reading frame 72 (*C9ORF72*) consisting of an intronic GGGGCC repeat expansion, and raised new hope that the molecular pathology of these devastating diseases could be unravelled. In 2012, more than 100 scientific articles followed up on this groundbreaking discovery.

The 2011 reports demonstrated that *C9ORF72* repeat expansions accounted for a large proportion of patients with familial ALS, as well as a subset of patients with sporadic ALS. To further assess this frequency, Majounie *et al.* screened more than 4,400 patients with ALS from 14 regions worldwide.[3] In patients with sporadic ALS, 7% of white individuals and 4% of African Americans were found to carry *C9ORF72* repeat expansions, whereas no such mutations were detected in comparatively small cohorts of Asian, Pacific Islander, Indian and Native American patients. Furthermore, an astonishing 38% of all studied patients with familial ALS had *C9ORF72* repeat expansions. These expansions were located in a shared disease-associated haplotype, suggesting a common disease 'founder' from whom these patients were descended. Numerous publications focusing on specific ALS populations corroborated these findings (Figure 1).[4]

ALS symptoms generally begin in the fifth or sixth decade of life, and approximately 25% of patients present with bulbar features.[5] The disease course is severely progressive, and most patients die within 3 years of onset. Among the few factors that predict a particularly poor prognosis are older age and bulbar onset. In his scientific commentary on

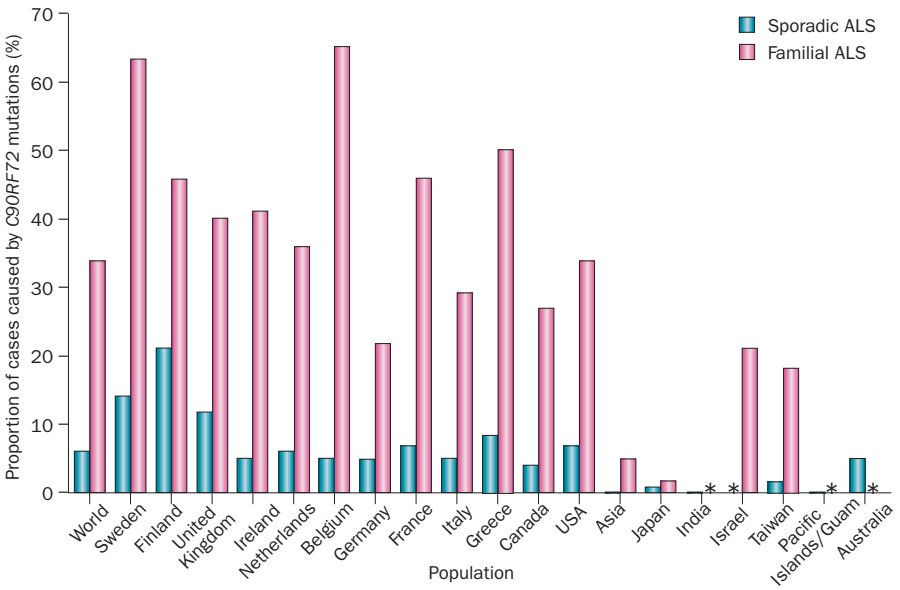

Figure 1 | Reported frequencies of *C9ORF72* mutations in patients with ALS. The mutation accounts for 34% of familial cases and 6% of sporadic cases worldwide. *Not determined. Abbreviations: ALS, amyotrophic lateral sclerosis; *C9ORF72*, chromosome 9 open reading frame 72.

eight studies, Hodges discussed the clinical characteristics of patients with *C9ORF72* expansions.[6] In these patients, bulbar onset seemed to be more common, and survival was significantly shorter compared with patients who did not harbour *C9ORF72* mutations. Moreover, up to 50% of patients with *C9ORF72*-related ALS demonstrated signs of cognitive impairment, and 30% were either diagnosed with dementia or had close relatives with this disease. Interestingly, some patients developed disease symptoms earlier than did their parents, which could indicate genetic anticipation—a phenomenon frequently described in repeat expansion disorders.

The presence of *C9ORF72* expansions in such a large percentage of patients within the diverse clinical spectrum of ALS and FTD suggests that other factors might influence disease phenotypes. In 2012, evidence for an oligogenic basis of ALS was indeed provided. Van Blitterswijk *et al.* assessed the mutation frequency of five major ALS-associated genes in a cohort of 97 families with ALS, and large cohorts of patients with sporadic ALS and control subjects.[7] The researchers identified five families with mutations in multiple genes—including two families with *C9ORF72* mutations—which is a significantly higher rate of mutation than would be expected by chance. One of these families contained a patient with a repeat expansion and an Asp90Ala mutation in superoxide dismutase 1 (*SOD1*), whereas the other family included patients with both *C9ORF72* expansions and Asn352Ser mutations in TAR DNA-binding protein 43 (*TARDBP*), which

Key advances

- Repeat expansions in chromosome 9 open reading frame 72 are an important cause of familial and sporadic amyotrophic lateral sclerosis (ALS) worldwide[1–4]
- Profilin 1 mutations are a novel cause of familial ALS[8]
- Ephrin type-A receptor 4 is a disease modifier of ALS[9]
- RNA lariat debranching enzyme is a modifier of toxicity associated with TAR DNA-binding protein 43[10]

encodes TDP43. In the latter family, the *C9ORF72* expansion was inherited from the father, whereas the *TARDBP* mutation was inherited from the mother. To date, more than 20 patients with GGGGCC expansions in *C9ORF72* and an additional mutation have been reported, strongly supporting an oligogenic pathogenesis.[4]

Despite the discovery of gene defects in *C9ORF72*, approximately half of familial ALS cases remained unexplained. To identify genes that could be implicated in these cases, Wu and colleagues performed exome sequencing in two families with dominantly inherited ALS.[8] The researchers identified two mutations in profilin 1 (*PFN1*), which encodes a key actin-binding protein. Successive sequencing of 272 patients with familial ALS revealed a total of four *PFN1* mutations in seven cases. Wu and colleagues then used *in vitro* models to demonstrate the presence of ubiquitinated insoluble aggregates that co-stained with TDP43 in 30–40% of PFN1-mutant cells. Mutant cells also displayed reduced actin binding relative to wild-type PFN1. In addition, mutated cells exhibited decreased axon length, reduced axon outgrowth, and altered growth-cone morphology. The findings highlighted the crucial role of cytoskeletal pathways in the pathogenesis of ALS, and emphasized that next-generation sequencing has the potential to elucidate the genetic underpinnings of many unresolved cases.

These discoveries all contribute to our understanding of ALS. To unravel pathogenic pathways and develop possible treatment strategies, however, additional *in vitro* and *in vivo* models have to be employed. Towards this end, two exciting studies published in 2012 used zebrafish and yeast models.

Van Hoecke and colleagues screened a library of 303 translation-blocking morpholinos in a zebrafish model of ALS that overexpressed mutant SOD1.[9] The investigators demonstrated that inhibition of Rtk2, the zebrafish homologue of human ephrin type-A receptor 4 (EPHA4), rescued the motor axonopathy associated with ALS. EPHA4 is a member of the ephrin subfamily of receptor tyrosine kinases, which have an important role in guiding axonal processes during migration, and seem to be implicated in neuronal maintenance and regeneration after injury. Importantly, Van Hoecke and colleagues showed that a 50% reduction in *Epha4* expression in $SOD1^{G93A}$ mice led to improved motor performance, reduced motor neuron degeneration, and prolonged survival. Moreover, in $SOD1^{G93A}$ rats, pharmacological inhibition of Epha4 delayed disease onset. Subsequent investigations extended these findings to patients with ALS.[9] Lower *EPHA4* mRNA levels, for example, were shown to correlate with a later disease onset and prolonged survival. Furthermore, direct sequencing of 192 patients with ALS identified two novel *EPHA4* mutations. These mutations were predicted to cause a loss of EPHA4 function, and were present in two patients with relatively long disease duration of more than 7 and 12 years. This study[9] illustrates how genetic screening in animal studies can be used to identify human disease modifiers and to provide novel avenues for clinical research in patients with ALS.

Armakola and colleagues performed two independent genome-wide deletion screens in a yeast model of TDP43 toxicity.[10] They identified deletion of lariat debranching enzyme (*DBR1*) as an effective suppressor of this toxicity. DBR1 catalyses debranching of RNA lariat introns—by-products of pre-mRNA splicing—and converts them into linear molecules for degradation. Armakola *et al.* showed that knockdown of *DBR1* in human neuroblastoma cells and primary rat neurons rescued toxicity associated with TDP43 overexpression. They also provided compelling data to support the hypothesis that, in the absence of DBR1, lariat introns accumulate in the cytoplasm and bind to TDP43, thereby preventing toxic interactions between TDP43 and other RNA molecules and RNA-binding proteins. Remarkably, *DBR1* deletion in yeast also suppressed toxicity associated with FUS, another RNA-binding protein implicated in ALS. *DBR1* deletion did not, however, suppress toxicity of α-synuclein or mutant huntingtin, suggesting a specific interaction between *DBR1* and ALS-associated proteins. This study provides a promising new route for development of effective therapies for TDP43-related and FUS-related proteinopathies, including ALS and FTD.

In summary, although ALS research in 2012 was dominated by studies focusing on the genetic and clinicopathological characterization of patients with newly discovered *C9ORF72* repeat expansions, the ongoing quest to determine the underpinnings of ALS also resulted in identification of novel ALS genes and genetic modifiers. In-depth research into each of these new players, and possibly their shared pathways, is expected to considerably improve our understanding of ALS pathobiology. These continuing efforts will in turn provide novel opportunities to develop therapeutic strategies for this fatal neurodegenerative disease.

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Competing interests
R. Rademakers holds a patent on methods to screen for the hexanucleotide repeat expansion in the *C9ORF72* gene. M. van Blitterswijk declares no competing interests.

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DEMENTIA IN 2012

Further insights into Alzheimer disease pathogenesis

Michael W. Weiner

In 2012, studies of autosomal dominant Alzheimer disease (AD), late-onset AD, and a rare genetic mutation of amyloid precursor protein provided support for the critical role of amyloid in AD pathogenesis. Increasing evidence implicated cell-to-cell transmission in the spread of tau and amyloid, highlighting novel targets for therapeutic intervention.

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2012 witnessed the failure of two amyloid-β (Aβ)-targeted drugs, bapineuzumab and solanezumab, in phase III trials for Alzheimer disease (AD)-related dementia. Such disappointing results have raised concerns about whether the treatments were initiated too late in the disease, whether Aβ levels were sufficiently reduced, and/or the validity of the 'amyloid cascade hypothesis', which places Aβ at the heart of AD pathology.

> "... the AD process begins more than 20 years prior to onset of dementia..."

Until now, much of the causal evidence for this hypothesis has come from studies of families with autosomal dominant AD, who have genetic mutations that lead to overproduction of Aβ. A recent paper by Bateman *et al.*[1] from the Dominantly Inherited Alzheimer's Network (DIAN) study is noteworthy, therefore, as it provides a comprehensive description of the sequence of clinical and biomarker changes that occur in autosomal dominant AD, enabling comparison with such changes in late-onset AD (LOAD)—the sporadic form that accounts for most AD patients. Bateman *et al.*[1] evaluated 88 carriers of mutations that cause autosomal dominant AD, and 40 healthy noncarriers. Clinical and cognitive parameters were assessed, as well as various brain scans and cerebrospinal fluid (CSF) levels of Aβ, tau and/or phosphorylated tau. Expected year of dementia onset (EYO) for each individual was estimated on the basis of the age of disease onset in their affected parent.

Differences in Mini-Mental State Examination score between mutation carriers and noncarriers were detected 5 years before EYO, and differences in memory function were detected 10 years before EYO. Decreased glucose metabolism in the precuneus in mutation carriers was detected 10 years before EYO, and hippocampal atrophy was detected 15 years before EYO. In addition, mutation carriers had substantial Aβ deposition in the precuneus and caudate nucleus 15 years before EYO—a pattern different to that seen in LOAD. Similar to studies of patients with LOAD, CSF tau was elevated 15 years before EYO, and CSF Aβ reached low levels 10 years before EYO, with concentrations seeming to decline as early as 25 years before EYO. Together, the findings of this study[1] suggest that the AD process begins more than 20 years prior to onset of dementia.

A limitation of the study by Bateman *et al.*[1] is that it used cross-sectional data to infer the longitudinal progression of events. Nevertheless, the finding that gene mutations that lead to overproduction of amyloid cause a sequence of biochemical and clinical changes similar to those seen in LOAD is consistent with the amyloid cascade hypothesis for LOAD.

Further evidence that the AD process begins several years prior to onset of dementia was provided by Buchhave *et al.*,[2] who found that CSF $A\beta_{1-42}$, but not tau, had reached disease-associated levels up to 10 years before onset of LOAD.

A landmark publication in 2012 by Jonsson *et al.*[3] added strong support to the amyloid cascade hypothesis through identification of a rare variant of the amyloid precursor protein (*APP*) gene that seems to protect carriers from AD. The investigators searched whole-genome sequence data from a large Icelandic cohort, including those aged at least 85 years without a diagnosis of AD, for *APP* mutations that affect AD risk. They found the single nucleotide polymorphism rs63750847—which results in an Ala673Thr substitution in APP—was significantly more common in elderly controls without AD than in the AD group (1/OR = 5.29), indicating strong protection against AD. In individuals aged 85, rs63750847 was enriched in those who were cognitively intact compared with those who had dementia (1/OR = 7.52). Furthermore, age-adjusted cognition was significantly better in carriers of rs63750847 than in noncarriers.

The Ala673Thr substitution on APP is located close to the target site of β-site APP cleaving enzyme 1 (BACE1)—the enzyme that generates Aβ, suggesting that the variant might impair BACE1 cleavage. Jonsson *et al.*[3] found that production of Aβ was reduced in cells transfected with Ala673Thr APP versus Aβ production in wild-type cells, and the mutant peptide was processed 50% less efficiently than wild-type peptide in a BACE1 cleavage assay. This study is the first example of a sequence variant conferring strong protection against AD, and indicates that reducing BACE1 cleavage of APP (that is, reducing production of Aβ) could protect against AD.

Despite considerable evidence for a causative role for Aβ in AD, focal neurodegeneration and symptomatology correlate most strongly with extent and amount of tau and/or phosphorylated tau deposition in the brain. Furthermore, memory impairment correlates with the level of tau-containing neurofibrillary tangles in the entorhinal cortex (ERC) and hippocampus.

The mechanism by which tau spreads from the ERC (possibly originating in the brainstem[4]) to the hippocampus and cortex has been a topic of great interest. The 2012 reports by Liu *et al.*[5] and deCalignon *et al.*[6] shed important new light on this issue. Both groups created a transgenic mouse model in which a mutant form of tau (linked to neurofibrillary tangle formation in frontotemporal dementia) was selectively expressed in a fraction of layer II neurons in the medial ERC (mERC). Using various staining techniques to detect misfolded tau pathology, they found production of misfolded tau in the mERC at

Key advances

- Studies of autosomal dominant Alzheimer disease (AD) suggest that the disease process begins more than 20 years prior to onset of dementia[1]
- A mutation in the gene encoding amyloid precursor protein that prevents cleavage into amyloid-β (Aβ) protects against late-onset AD[3]
- Misfolded tau seems to be transferred from neuron to neuron[5,6]
- Inoculation of mouse brains with Aβ produced widespread cerebral amyloidosis[7]

> “...research highlights of 2012 provide new support for the central role of amyloid in AD pathogenesis”

3–6 months of age. Mutant tau pathology was found months later in the granular layer of the dentate gyrus, followed by its accumulation in hippocampal subfields, and then in the cortex. Given that the mutant tau was only expressed in the mERC and not in ‘downstream’ regions, the pattern of spread suggested that misfolded tau is transferred to new cell populations, recapitulating the tauopathy that defines early stages of AD. These results also highlight a potential approach to therapy that targets cell-to-cell transmission of tau.

Along similar lines, Stohr *et al.*[7] investigated cell-to-cell transmission of Aβ through inoculation of mouse brains with either purified Aβ from brain aggregates or with synthetic Aβ aggregates. The intervention was found to cause widespread cerebral amyloidosis, leading the authors to conclude that Aβ alone is sufficient for formation for a self-propagating protein assembly, and that “Aβ aggregates are prions.” Indeed, reviewing evidence for cell-to-cell spread of Aβ, tau, α-synuclein, superoxide dismutase, and huntingtin protein, Prusiner[8] proposed that “proteins causing neurodegeneration are all prions.”

Despite these advances, the mechanism by which Aβ and tau interact to cause AD remains poorly understood. Accumulation of Aβ in transgenic mice does not produce the characteristic tauopathy, and in human frontotemporal dementia and chronic traumatic encephalopathy, widespread deposition of misfolded tau occurs without accumulation of Aβ. Nevertheless, the important findings reported in 2012 concerning cell-to-cell transmission of tau and Aβ certainly suggest new targets for therapy.

In conclusion, despite disappointing results from clinical trials of anti-amyloid monoclonal antibodies, the research highlights of 2012 provide new support for the central role of amyloid in AD pathogenesis. In addition, the growing number of papers reporting cell-to-cell transmission of tau, amyloid and other misfolded proteins highlight an exciting area that will lead to improved understanding of the mechanisms by which these proteins cause neurodegeneration and lead to clinical symptoms, and could provide targets for development of new therapeutics.

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Competing interests
The author declares an association with the following company: Elan. See the article online for full details of the relationship.

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EPILEPSY IN 2012

Advances in epilepsy shed light on key questions

Ingrid E. Scheffer and Saul A. Mullen

Research on epilepsies in 2012 has substantially advanced our knowledge of these often devastating conditions. From important discoveries that revealed causative factors and the molecular basis of disease, to major implications for surgical decision-making, these studies set the scene for future advances in the field.

Scheffer, I. E. & Mullen, S. A. *Nat. Rev. Neurol.* **9**, 66–68 (2013); published online 8 January 2013;
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A common and often devastating group of disorders, the epilepsies deserve an intense research focus to improve our understanding of their neurobiology and, thereby, improve the lives of affected individuals. In 2012, some studies in this field employed prospective large-cohort designs to answer key questions, whereas others yielded insights into the molecular determinants of both mild and severe epilepsies, as well as cortical malformations.

Therapeutic epilepsy surgery, although undeniably effective in lesional epilepsies, has been applied late and inconsistently in most patients. Publishing their results in 2012, the Early Randomized Surgical Epilepsy Trial (ERSET) study group compared surgical therapy with continued medical therapy in patients with refractory mesial temporal lobe epilepsy (MTLE)—an epilepsy in which surgery generally carries a good prognosis.[1] Participants had MTLE that was well-localized in terms of both imaging and EEG, and had failed two adequate trials of antiepileptic drugs. A unique feature of this trial is that participants were included within 2 years of failing their medication trial, which enabled comparison of surgery with medical therapy at the earliest point that patients could be deemed ‘refractory’. The researchers hypothesized that early, successful treatment would minimize both long-term comorbidities and risk of premature death.[2] Surgery early in the course of epilepsy, however, was still regarded as so radical that the trial was unable to recruit adequate numbers of participants and was stopped prematurely, with only 38 of the planned 200 participants included.

Despite recruitment difficulties, the trial was overwhelmingly positive: seizure freedom at 2 years was achieved in 73% of surgically treated patients ($n = 15$) compared with none of the medical therapy group ($n = 23$).[1] Disabling seizures were defined by the presence of objective features, impaired function or awareness, or convulsive attacks. Isolated auras were not included in the assessment. Drawing of conclusions with regard to the secondary outcomes, such as quality of life, was difficult owing to the small sample size, but a trend towards improved quality of life

Key advances

- Temporal lobe surgery should be considered early in cases of mesial temporal lobe epilepsy when the patient has failed to respond to two antiepileptic drugs[1]
- Febrile status epilepticus is associated with mesial temporal lobe changes on early MRI in 10% of cases, and underlying hippocampal malrotation may be a predisposing factor for this disorder[5]
- Benign familial infantile epilepsy and infantile convulsions with choreoathetosis are due to mutations in *PRRT2*, molecularly linking movement disorders and epilepsies that present at different ages[6–8]
- The long-held theory that somatic mutations cause cerebral malformations was proven with the discovery of mosaic somatic mutations in genes linked to hemimegalencephaly[9,10]

following surgery was observed, with more individuals from the surgical group than from the medical therapy group able to drive and socialize after treatment. However, compared with those who received medical therapy, patients in the surgical group showed a trend towards greater decline in verbal recall and naming.

In summary, the ERSET study emphasizes that epilepsy surgery can lead to dramatic improvements in seizure control and probably also in psychosocial outcome, and should be strongly considered early in the course of refractory MTLE.

Other key studies in epilepsy in 2012 dealt with causative factors, such as the link between febrile seizures and TLE with hippocampal sclerosis. Although retrospective studies have long suggested an association between febrile status epilepticus (FSE) and subsequent TLE, epidemiological studies of febrile seizures have, as a whole, failed to confirm this association.[3,4] The FEBSTAT (Consequences of Prolonged Febrile Seizures in Childhood) study—a large prospective study of 199 children with FSE—used early MRI investigations to show acute changes and subtle underlying brain malformations that may predispose patients to hippocampal injury.[5] Over 10% of children with FSE had increased hippocampal T2 signal on early MRI (that is, within 1 week of presentation) that was suggestive of hippocampal oedema. This phenomenon was not seen in a control cohort of children with simple febrile seizures. Patients with FSE were also more likely to have an underlying developmental anomaly of the hippocampus, which was predominantly incomplete rotation of the hippocampus.

Long-term follow-up of patients included in the FEBSTAT study promises to yield answers to questions such as whether acute hippocampal changes predict the evolution of hippocampal sclerosis and MTLE. For now, this trial confirms that acute temporal lobe injury occurs in a substantial group of children with FSE, and raises questions about an underlying, predisposing developmental lesion.

Major technological advances in molecular biology are reaping rewards in epilepsy. After 20 years, the molecular basis of the well-recognized autosomal dominant syndrome benign familial infantile epilepsy, and of the related entity of infantile convulsions with choreoathetosis, has been solved with the use of whole-exome sequencing. Affected individuals present with seizures in infancy and/or paroxysmal kinesigenic dyskinesia that begin from childhood to young adult life. Mutations in the gene that encodes proline-rich transmembrane protein 2 (*PRRT2*) were found in 80% families affected with this disorder—an observation confirmed by many groups worldwide.[6,7] Little is known about PRRT2 other than its interaction with a protein involved in membrane docking and calcium-triggered neuronal exocytosis. Numerous recent studies showed that migraine of many types, including hemiplegic migraine and migraine with or without aura, are also manifestations of *PRRT2* defects.[8] These studies of *PRRT2* mutations highlight that one protein can cause epilepsy, movement disorders and migraine, and—in biological terms—can tie together these paroxysmal neurological disorders with different ages of onset and different anatomical substrates.

Molecular studies have elegantly demonstrated that somatic mutations of genes that are critical to brain development may result in the cerebral malformation hemimegalencephaly. Patients with this rare disorder, which comprises an enlarged malformed cerebral hemisphere, often undergo epilepsy surgery, thereby permitting removal of cerebral tissue for use in molecular studies. In one study, Poduri *et al.*[9] noted that, of eight hemimegalencephalic specimens studied, two had mosaic partial trisomy 1 that encompassed many genes, with the notable inclusion of *AKT3*—a gene that encodes a protein kinase involved in control of brain size. Sequencing of *AKT3* in the remaining six patients identified a mosaic, activating point mutation in one case. Blood leukocytes from two patients with cerebral *AKT3* mutations did not exhibit the genetic anomaly, confirming that the mutation was indeed somatic.

Many lines of evidence converge to support *AKT3* as the underlying molecular determinant of hemimegalencephaly. However, as only three of eight cases had mosaic mutations involving *AKT3*, this anomaly seems to represent the molecular basis of disease in only a subset of patients with hemimegencephaly. What about the molecular basis of the remaining cases?

In another study of patients with the cerebral malformation hemimegalencephaly, Lee *et al.*[10] identified *de novo* somatic mosaic gain-of-function mutations in six of 20 cases, and the results implicate several genes—namely, *PIK3CA*, mammalian target of rapamycin (*MTOR*) and *AKT3*—in this disorder. Interestingly, these genes encode regulators of the mTOR signalling pathway, which is involved in cell growth and metabolism. This work suggests that other cerebral malformations could have a somatic and potentially mosaic mutational basis, and sets the scene for future research in this area.

> “Major technological advances in molecular biology are reaping rewards in epilepsy”

Although differing in focus and method, these papers all advance understanding of epilepsy. The ERSET trial[1] has fundamentally altered how we think about epilepsy surgery, and the FEBSTAT study[5] should, once the final data are available, answer fundamental questions on the aetiology of temporal lobe epilepsy. Such large-scale, rigorous prospective studies on important questions are vital in advancing therapeutics in epilepsy. By contrast, the findings in benign familial infantile epilepsy [6–8] and hemimegalencephaly[9,10] highlight that small-scale targeted science as well as family-scale genetic studies can still lead to landmark discoveries. These approaches set the scene for massive parallel sequencing studies of large cohorts that will radically change our understanding of epilepsy in 2013.

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STROKE IN 2012

Major advances in the treatment of stroke

Miguel Blanco and José Castillo

Several clinical trials and experimental studies that could have a major impact on the treatment of patients with ischaemic stroke were published in 2012. The studies cover all therapeutic options, including stroke prevention, recanalization and thrombolysis, neuroprotection, and promising new therapeutic approaches focused on neurorepair.

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Despite improvements in its management in recent years, cerebrovascular disease remains a major cause of mortality and morbidity. The current framework of therapeutic options to prevent or treat stroke comprises a spectrum of five fields of treatment—primary prevention, recanalization and thrombolysis, neuroprotection, secondary prevention, and neurorepair (Figure 1). In 2012, we witnessed developments in all five areas, with important implications for stroke treatment.

When primary prevention fails, an effective protocol for acute stroke treatment is imperative, early reperfusion of ischaemic tissue being the primary goal. Currently, thrombolytic therapy with recombinant tissue plasminogen activator (rtPA) is the most effective approach. However, the extensive exclusion criteria, together with the short therapeutic window and narrow age range, severely restrict its use. The Third International Stroke Trial (IST-3)[1] was conducted to establish the balance between benefits and harms of rtPA treatment in patients who did not accomplish the licence criteria and, hence, to determine whether a wider range of patients might benefit up from rtPA up to 6 h from stroke onset.

IST-3 enrolled 3,035 patients (1,515 in the rtPA group and 1,520 controls), 53% of whom were aged >80 years. No differences were found between groups with respect to the primary end point (37% in the rtPA group versus 35% of controls survived and achieved independence). The global benefits of rtPA treatment seemed to be greatest within the first 3 h from stroke onset, but the analyses had insufficient power to define the benefit in a therapeutic window beyond 3 h. Interestingly, treatment efficacy was similar between patients older and younger than 80 years. The IST-3 data reinforce the need for further efforts to increase the proportion of ischaemic strokes treated within 3 h, but they provide reassurance that rtPA treatment in elderly patients and within 6 h from stroke onset does not increase mortality.

Although clinical trials with neuroprotective agents have systematically failed, neuroprotection remains a treatment option for acute ischaemic stroke. In 2012, the results of ICTUS (International Citicoline Trial on acUte Stroke) were published.[2] In this trial, patients with moderate to severe acute ischaemic stroke were treated with either citicoline or placebo within 24 h after symptom onset. The primary outcome was recovery at 90 days measured by a global test combining three measures—NIH Stroke Scale score ≤1, Modified Rankin Scale score ≤1, and Barthel Index score ≥95—in accordance with a pooled meta-analysis of previous randomized trials with citicoline.[3]

2,298 patients (1,148 assigned to citicoline and 1,150 to placebo) were enrolled in ICTUS.[2] Global recovery was similar in both groups, and no significant differences were reported in the safety variables or the rate of adverse events. Although ICTUS followed a protocol nearly identical to that of the pooled meta-analysis,[3] with minimal differences in statistical analysis, none of the benefits for citicoline were confirmed. However, the previous clinical trials with citicoline were conducted 10 years ago, and the standard of stroke care has substantially improved in the meantime. Also, the patients in ICTUS were, on average, 4 years older, and were over three times more likely to have received rtPA treatment, than those in previous trials. Comparison with the older studies suggests that the benefits of citicoline have become diluted in parallel with improvements in the standard of care for acute ischaemic stroke—a fact that should be taken into account for future trials of neuroprotective drugs.

To initiate appropriate secondary prevention, it is essential to know the cause of stroke, yet at least one-quarter of strokes are still classified as cryptogenic. The ASSERT investigators recruited 2,580 individuals >60 years without a history of atrial fibrillation (AF) in whom a pacemaker or defibrillator had been implanted.[4] Patients were

monitored for 3 months to detect subclinical atrial tachyarrhythmias (ATs; episodes of atrial rate >190 bpm for >6 min), and were followed up for a mean of 2.5 years for the primary outcome of ischaemic stroke or systemic embolism. At least one AT was detected in 10.1% of patients. During the follow-up period, 4.2% of patients in whom subclinical AT had been detected had an ischaemic stroke or systemic embolism, compared with 1.7% in whom subclinical AT was not detected. The attributable risk of ischaemic stroke or systemic embolism associated with subclinical AT was 13%, which is similar to the stroke risk associated with AF.[5] On the basis of these results, AT must be considered as a new source of embolism in patients with cryptogenic stroke.

The prevalence of patent foramen ovale (PFO) ranges from 20–26% in the general population, but may be as high as 56% in patients under 55 years who have experienced a cryptogenic stroke.[6] The CLOSURE I investigators evaluated the potential benefits of percutaneous device closure versus medical therapy for secondary stroke prevention in patients with PFO.[7] This multicentre, randomized, open-label trial compared PFO closure (using the STARFlex device) with medical therapy (warfarin, aspirin or both) in patients 18–60 years of age with PFO who had presented with cryptogenic stroke or TIA within the previous 6 months. The primary end point was a composite of stroke or TIA during 2 years of follow-up, death from any cause during the first 30 days, or death from neurological causes between 31 days and 2 years.

909 patients—447 percutaneous device and 462 medical therapy—were enrolled. At 2 years, effective closure was maintained in 86.7% of cases. The incidence of the primary end point was 5.5% in the percutaneous device group and 6.8% in the medical group. Stroke incidence was 2.9% in the percutaneous device group and 3.1% in the medical group. No deaths occurred by 30 days in either group, and there were no deaths from neurological causes during the 2-year follow-up period. AF was significantly more frequent in the closure group (5.7% versus 0.7%). In conclusion, closure did not offer greater benefits than medical therapy alone for the prevention of recurrent stroke or TIA.

Over the past two decades, stem cell-based neurorepair has emerged as a promising therapeutic option for ischaemic stroke. Animal experimental data with embryonic stem (ES) cells, neural progenitor cells or bone marrow-derived progenitor cells are

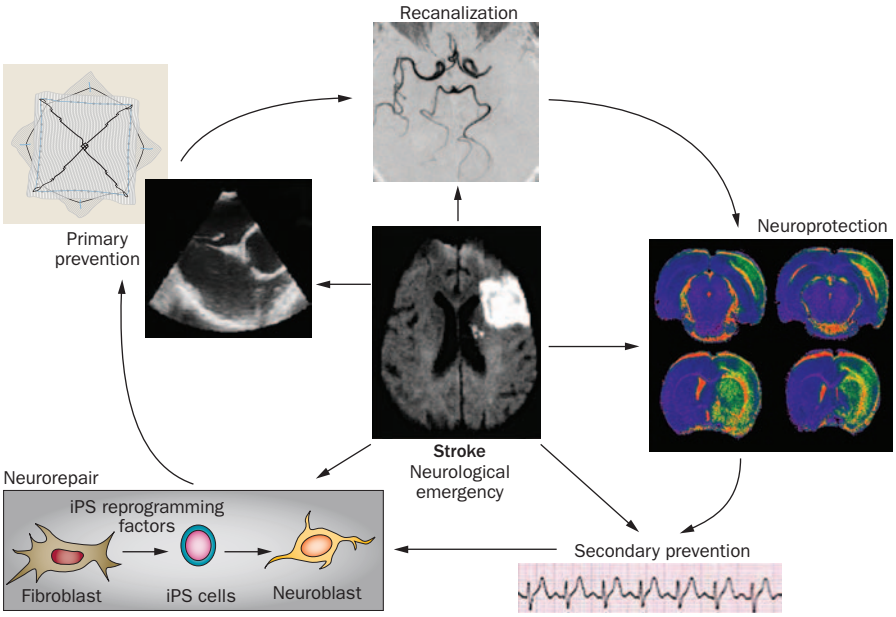

Figure 1 | Spectrum of therapeutic options to prevent or treat stroke. In 2012, key advances were made in stroke prevention (primary and secondary), recanalization and thrombolysis, neuroprotection, and neurorepair strategies. Abbreviation: iPS, induced pluripotent stem.

encouraging; however, no proven stem cell-based therapy is currently available for stroke. Despite the supposed immunoprivileged status of the CNS, allogeneic grafts of stem cell-derived neurons and glia remain susceptible to rejection. A novel alternative strategy to avoid graft rejection and immunosuppression is to generate induced pluripotent stem cells (iPSCs) from somatic cells.

In 2006, it was reported that skin fibroblasts from adult mice could be reprogrammed to a pluripotent state by retroviral expression of four transcription factors.[8] The resulting iPSCs are indistinguishable from ES cells in morphology, proliferative capacities, surface antigens, gene expression, epigenetic status, and telomerase activity. The Nobel Prize in Physiology or Medicine 2012 was awarded to Sir John B. Gurdon and Shinya Yamanaka for this breakthrough.

The Laboratory of Neural Stem Cell Biology and Therapy, Lund, Sweden transplanted long-term self-renewing neuroepithelial-like stem cells, generated from adult human fibroblast-derived iPSCs, into stroke-damaged mouse and rat striatum or cortex.[9] The transplanted cells stopped proliferating, could survive without forming tumours for at least 4 months, and differentiated into morphologically mature neurons. Grafted cells exhibited electrophysiological properties of mature neurons and received synaptic input from host neurons. Most importantly, recovery of forepaw movements was observed by 1 week after transplantation. This study provides the first evidence that transplantation of human iPSC-derived cells is a safe and efficient approach to promote repair and recovery after stroke.

The advances described above reflect progress across the spectrum of therapeutic options in stroke, from primary prevention to neurorepair (Figure 1). Every step takes us towards the ultimate aim of better outcomes and quality of life for patients with stroke.

Key advances

- IST-3 reinforces the need for further efforts to increase the proportion of ischaemic strokes treated within 3 h, but provides reassurance that thrombolysis in elderly patients or within 6 h from stroke onset does not increase mortality[1]
- Under the conditions of the ICTUS trial, citicoline is not effective in the treatment of moderate to severe acute ischaemic stroke[2]
- Atrial tachyarrhythmia must be considered as a new source of embolism in patients with cryptogenic stroke[4]
- Patent foramen ovale closure with a device did not offer a greater benefit than medical therapy alone for the prevention of recurrent stroke or TIA[7]
- An animal study provides the first evidence that transplantation of cells derived from human induced pluripotent stem cells is a safe and efficient approach to promote recovery after stroke[9]

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J. Castillo declares an association with the following company: Ferrer Grupo. See the article online for full details of the relationship. M. Blanco declares no competing interests.

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MOVEMENT DISORDERS IN 2012

Advancing research towards novel therapeutic approaches

Nikolaus R. McFarland and Michael S. Okun

Research in movement disorders in 2012 has improved our understanding of the pathogenic mechanisms of disease and led to development of potential novel therapeutic approaches. Key advances were linked to mechanisms underlying spread of neurodegenerative pathology, immunotherapy, stem cells, genetics and deep brain stimulation in parkinsonism and related disorders.

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Despite recent advances in our understanding of the pathogenesis and treatment of movement disorders such as Parkinson disease (PD), many of these syndromes remain challenging to diagnose and to treat. Moreover, disease-modifying therapies remain elusive. Continued progress in elucidating the pathophysiology of these disorders and translation of research from bench to bedside will result in better diagnostics and development of novel therapeutics. Research in 2012 has moved the field closer to this goal, and important progress had been made in numerous areas, including basic and clinical work in parkinsonism, dystonia, Huntington disease (HD), essential tremor, and tic disorders. We highlight some of the recent and key advances in each area that are collectively leading to improved understanding of basic pathophysiology and novel approaches to therapy.

A major hallmark of PD and related disorders is the presence of Lewy body pathology, with concomitant neurodegeneration linked to abnormal accumulation and deposition of α-synuclein. Mounting evidence supports the hypothesis of prion-like spread of pathology via cell-to-cell transmission of pathological forms of proteins such as α-synuclein.[1] Unanswered questions remain, however, about the mode of transmission of pathological α-synuclein species and their role in disease pathogenesis.

Building on previous work demonstrating the ability of preformed α-synuclein fibrils to precipitate Lewy body-like pathology and neurodegeneration, Luk and colleagues recently showed that intracerebral injections of exogenous preformed fibrils or brain homogenates from old, symptomatic Ala53Thr transgenic mice (which express Ala53Thr human α-synuclein) into younger, asymptomatic mice could accelerate Lewy pathology.[2] In the inoculated animals, widespread α-synuclein pathology—including misfolded and hyperphosphorylated α-synuclein and intracellular Lewy-like inclusions—was observed, with a resultant decrease in survival of these animals. The findings provide further evidence of prion-like spread and propagation of synucleinopathy, and cell-to-cell transmission of pathological proteins. Furthermore, exogenous preformed α-synuclein fibrils were sufficient to produce this cascade of events and accelerate the disease phenotype *in vivo*. On the basis of these findings, targeting of cell-to-cell transmission to block the spread of synucleinopathy is a promising therapeutic approach to disorders such as PD.

Evidence points to an important role for release of extracellular α-synuclein from neurons in the transmission of pathology. Approaches such as passive and active immunization to target α-synuclein have shown promise in synucleinopathy models, with reduction in α-synuclein accumulation and associated neurodegeneration.[3,4] The exact mechanism of action, however, remains unclear. In a study published in *The Journal of Neuroscience*, Bae *et al.* hypothesized that antibodies against α-synuclein target extracellular α-synuclein and aid microglia in the clearance of pathological protein species, thereby preventing cell-to-cell propagation of pathology.[5] The researchers showed that α-synuclein antibodies bound to extracellular aggregates are taken up by microglia through surface Fcγ receptors, and are then delivered to microglial lysosomes for degradation. Intracerebral injection of α-synuclein antibody in a transgenic mouse model also resulted in α-synuclein clearance, and specifically reduced neuron-to-astroglia transmission of the protein, promoting microglial uptake and removal of extracellular α-synuclein. These studies help to further elucidate the mechanism of cell-to-cell transmission of α-synuclein, and highlight a potential immunotherapy for PD and related disorders.

Advances in other synucleinopathies such multiple system atrophy (MSA)—a disorder in which standard PD therapies often fail—have also been made. Following an initial open-label trial of autologous mesenchymal stem cells (MSCs) in MSA that attracted criticism from researchers in the field but also provided hope for a novel therapy, Lee *et al.* reported the results of a 1-year randomized clinical trial.[6] The therapeutic mechanism

of MSCs in neurodegenerative disease is debated, but these cells are known to be capable of differentiating into various cell types under specific conditions, and also of secreting potentially neuroprotective trophic factors. In this study, vehicle or MSCs were administered to patients intra-arterially followed by thrice-monthly intravenous infusions of the cells. 27 of the 33 enrolled patients with MSA completed the study, and all participants demonstrated delayed progression on the Unified MSA Rating Scale (UMSARS). However, no difference was observed in the UMSARS daily living scores between the two groups, indicating that the treatment predominantly affected motor deficits.

Secondary outcomes, including functional (PET) and structural (MRI) brain imaging, also indicated beneficial effects of MSC therapy.[6] Although encouraging, this study had several limitations. It was performed in a single centre and included only patients with cerebellar-predominant MSA, which prevents extrapolation to parkinsonism-predominant MSA—a form of MSA that is more-frequently encountered by physicians in the USA, Canada and Europe. Importantly, one-third of patients who received either vehicle or MSCs had ischaemic lesions on MRI, raising the issue of intervention-associated risks. The transiency of the therapeutic effects, however, indicated the need for repeated administration. Although these major concerns must be resolved for future application of MSCs in clinical practice, this study clearly highlights a potential novel therapeutic approach to MSA, and possibly to other neurodegenerative disorders.

Induced pluripotent stem cells (iPSCs) have potential to become a powerful therapy for neurodegenerative diseases. Patient-derived iPSCs provide a source of stem cells for autologous transplantation and avoid the risk of rejection associated with allogenic transplants. The main drawback of this therapy is that patient-derived stem cells harbour the disease-causing mutation. An *et al.* performed a proof-of-concept experiment in which the CAG repeat expansion that causes HD was corrected in patient-derived iPSCs.[7] Fibroblasts isolated from patients with HD were reprogrammed into iPSCs, and the HD repeat expansion was corrected by insertion of a normal copy of the Huntingtin gene and stimulation of homologous recombination.

The molecular phenotypes associated with HD—including defects in apoptosis, cell signalling and bioenergetics, and abnormal levels of brain-derived neurotrophic factor—were reversed in the genome-corrected cells, but remained in the uncorrected iPSCs.[7] Importantly, the genome-corrected iPSCs retained pluripotency, differentiating not only into neural stem cells but also into striatal neurons *in vitro* and *in vivo*. Strikingly, when transplanted into the striatum of the R6/2 mouse model of HD, neural stem cells derived from the genome-corrected iPSCs populated the striatum and differentiated into γ-aminobutyric acid-releasing medium spiny neurons and astrocytes. Whether the iPSCs could reverse the behavioural deficits and early lethality seen in the R6/2 mouse was not reported. This study highlights the utility and feasibility of patient-derived iPSCs as a future therapeutic option and has far-reaching implications not only for HD, but also for all neurodegenerative disorders that are underpinned by a known gene mutation.

Deep brain stimulation has revolutionized therapy for PD and dystonia, and is being increasingly considered for use in other conditions such as Tourette syndrome. In a recent study, patients with highly medically resistant Tourette syndrome were implanted with centromedian thalamic deep brain stimulation electrodes. In a series of studies, the investigators showed that scheduled rather than chronic stimulation may suppress tics.[8] More importantly, however, was the observation of a clinical correlation between the presence of γ-band activity and decreased tic severity. Temporal correlation between the power of γ-band activity and tic suppression was evident. In PD, β-band activity has emerged as an important physiological marker of disease.[9] In Tourette syndrome, modulation of γ-band activity could be critical to the success of new therapeutics.[9,10]

Together, the advances highlighted here demonstrate continued progress in understanding the pathological mechanisms of movement disorders and the critical nature of these findings in developing novel therapeutics. Translation of basic findings into new approaches to target these diseases will be essential to advance future therapies.

Key advances

- Inoculation of the brain with pathological α-synuclein results in rapidly progressive accumulation and seeding of endogenous α-synuclein, and neurodegenerative pathology[2]
- Administration of α-synuclein-specific antibodies prevents neuronal transmission of α-synuclein and promotes microglial uptake and degredation of this protein, supporting extracellular α-synuclein as a potential therapeutic target in Parkinson disease[5]
- Mesenchymal stem cell therapy in multiple system atrophy represents an exciting potential therapeutic advance but requires further study[6]
- Correction of the genetic defect (an expanded CAG repeat) in Huntington disease can be achieved in pluripotent stem cells derived from patients, and reverses associated disease pathology in neural stem cells[7]
- Scheduled rather than chronic deep brain stimulation may suppress tics in Tourette syndrome and correlates with γ-band activity[8]

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MULTIPLE SCLEROSIS IN 2012

Novel therapeutic options and drug targets in MS

Axel Methner and Frauke Zipp

2012 witnessed important developments for multiple sclerosis, including successful phase III trials of novel oral therapeutics and identification of the potassium channel KIR4.1 as an autoimmune target. Additionally, the lung was highlighted as an important site for immune-cell programming, and the relevance of a TNF receptor variant was clarified.

Methner, A. & Zipp, F. *Nat. Rev. Neurol.* **9**, 72–73 (2013); published online 22 January 2013; doi:10.1038/nrneurol.2012.277

Multiple sclerosis (MS) is a disabling demyelinating autoimmune disorder that mainly affects young adults. Disability in patients with MS is mainly due to neuronal pathology, which is at best only partially amenable to current immunomodulatory therapy. Two novel oral therapeutics, BG12 (dimethyl fumarate) and laquinimod, promise to tackle both the aggressive autoimmune response against myelin and the neuronal pathology that occurs in MS. Results of three phase III clinical trials describing the efficacy of these drugs in relapsing–remitting MS (RRMS), the early inflammatory phase of MS, were published in 2012 in *The New England Journal of Medicine*.[1–3]

The trial reported by Fox *et al.*[1] involved 1,417 patients with RRMS, who were randomly assigned to receive 240 mg BG12 two or three times daily, placebo or the approved immunomodulatory agent glatiramer acetate. The primary end point was the annualized relapse rate (ARR) over a 2-year period, which was significantly lower with 480 mg BG12 (ARR = 0.22), 720 mg BG12 (ARR = 0.20) or glatiramer acetate (ARR = 0.29) compared with placebo (ARR = 0.40). All drugs significantly reduced the number of new or enlarging T2-weighted hyperintense lesions and new T1-weighted hypointense lesions on MRI. Adverse events, mainly flushing and gastrointestinal events for BG12 (which usually resolved spontaneously over time) and injection-related events for glatiramer actetate, were more prevalent in the treatment groups than in the placebo group.

A significant reduction in disability progression was not achieved in the trial by Fox *et al.*,[1] even with the approved drug glatiramer acetate, but was achieved in the placebo-only controlled study of BG12 by Gold *et al.*[2] The main reason for this difference was presumably the unexpectedly low rate of progression in the placebo group in the Fox *et al.*[1] study. The study by Gold *et al.*[2] involved 1,234 patients with RRMS, and found that progression of disability was 27% in the placebo group, 16% in the 480 mg BG12 group, and 18% in the 720 mg BG12 group, which corresponded to a relative risk reduction of 38% and 34%, respectively.

> “Identification of new targets ... opens new avenues that will increase our therapeutic portfolio”

The trial of laquinimod reported by Comi *et al.*[3] included 1,106 patients with RRMS who received either 0.6 mg oral laquinimod once daily or placebo for 24 months. The primary end point was the ARR over 24 months, which was reduced in the treatment group compared with placebo (0.30 versus 0.39, $P = 0.002$). Interestingly, differences in the secondary outcome parameters of disability progression and brain volume change between the laquinimod and placebo groups were more pronounced than differences in ARR or the number of gadolinium-enhancing lesions, which could be interpreted as evidence of an additional, neuroprotective property of laquinimod. Adverse events were mainly restricted to clinically irrelevant transient elevations of alanine aminotransferase levels.

These trials[1–3] support the efficacy of novel therapeutic options with different adverse effect profiles to those currently on the market, providing a wider choice of oral drugs to patients. The trials were not, however, designed to evaluate the effect of the drugs on the secondary neurodegenerative phase of MS, which is mainly responsible for chronic disability progression—a major unsolved clinical problem in this disease.

Understanding of mechanistic drug effects is mainly derived from studies using the animal model of MS: experimental autoimmune encephalomyelitis (EAE). Previous studies in this model showed that BG12 exerts neuroprotective effects against inflammation by activating the nuclear factor erythroid 2-related factor 2 (NRF2)[4]—a transcription factor that induces expression of genes involved in the antioxidant response. Building on this work, we showed in 2012 that BG12 concentrations that inhibit anti-inflammatory cytokine production also reduce cell death caused by oxidative stress and do not alter neuronal network activity in dissociated neuronal cultures.[5] These findings suggest that BG12 might target the dysregulated immune system and the neurodegenerative pathology of MS.

Laquinimod has been shown to reduce inflammatory cell infiltrates in the brain and decrease demyelination and axonal loss in EAE.[6] Studies in 2012 suggest that laquinimod modulates adaptive T-cell immune responses via cells of the innate immune system,[7] possibly by interference with nuclear factor κB (NF-κB) signalling (A. Waisman, personal communication; F. Zipp, unpublished work). The transcription factors NRF2 and NF-κB thus constitute novel MS drug targets that came into focus in 2012.

In another study published in *The New England Journal of Medicine*, Srivastava *et al.*[8] screened serum IgG for binding to brain tissue preparations, and observed specific antibody binding to glial cells in 46.9% of patients with MS, versus 0.9% of patients with other neurological diseases, and no binding in healthy donors. The antigen was identified as the ATP-sensitive inwardly rectifying potassium channel KIR4.1. Co-injection of patient-derived KIR4.1-specific IgG and human complement into the cisternae magnae of mice resulted in loss of KIR4.1 expression on astrocytes and oligodendrocytes, which suggests that the immune response against KIR4.1 can induce structural damage to glial cells. Given that KIR4.1 knockdown in rat astrocytes impairs potassium buffering and glutamate uptake, pathological downregulation of this channel could conceivably cause neuronal degeneration through excitotoxic overstimulation of ionotropic glutamate receptors or depletion of the antioxidant glutathione by excess glutamate. Autoantibodies against KIR4.1 were also detected in the cerebrospinal fluid in 19 of 30 patients with MS. In two of these patients, antibody synthesis occurred intrathecally, meaning that the antibodies would not have

to cross the blood–cerebrospinal fluid barrier to reach their target.

Another MS drug target, and indeed a novel target organ, was reported by Odoardi *et al.*[9] in *Nature*. In this intriguing study, the authors showed that the lung is the anatomical site in which T cells develop a more migratory phenotype and are enabled to enter the CNS. The EAE disease course was ameliorated by trapping T cells in this compartment, thereby blocking their entry into the CNS, through various approaches: for example, using the oral MS therapeutic FTY720 or by blocking proteins such as integrins (the target of the MS therapeutic natalizumab) or the adhesion protein Ninjurin 1 that are upregulated on T cells during their transit through the lung and facilitate entry to the CNS. A more detailed analysis of proteins and pathways that mediate the migratory phenotype of pathogenic T cells will be instructive for the identification of additional drug targets for future studies.

Another important development in the MS field in 2012 was provided by Gregory *et al.*,[10] who focused on a single nucleotide polymorphism in the gene encoding tumour necrosis factor receptor 1 (TNFR1) that is associated with MS but no other autoimmune diseases. The researchers showed that this polymorphism causes exon skipping and expression of a soluble truncated form of TNFR1 that can block TNF and, thereby, mimic the effect of TNF-blocking drugs. This finding is instructive, as although inhibition of TNF is common practice in the treatment of several autoimmune diseases, a phase III trial of an antagonistic soluble TNF receptor in patients with MS had to be stopped as the treatment exacerbated disease. In the upcoming era of personalized medicine, connecting patient genetic variation with results from genome-wide association studies and drug effects will be important for optimal patient management.

Key advances

- Novel oral therapeutics for relapsing–remitting multiple sclerosis (MS), which promise immunomodulatory and neuroprotective activity, successfully completed phase III trials[1,3]
- Potassium channel KIR4.1 is a novel autoantigen in MS[8]
- A 'stopover' in the lung is necessary to enable T cells to invade the brain[9]
- Some patients with MS express a variant tumour necrosis factor (TNF) receptor, which might underlie the unexpected failure of TNF-blocking agents in MS treatment[10]

In conclusion, 2012 added novel oral treatment options for MS that promise a dual effect on inflammation and neurodegeneration. Identification of new targets such as KIR4.1 and proteins involved in regulation of the T-cell migratory phenotype acquired in the lung—an organ that was not on the landscape of immunologists and neurologist—opens new avenues that will increase our therapeutic portfolio in the fight against MS. Finally, personalized medicine approaches are on the horizon that will enable identification of patients who stand to benefit most from the arsenal of MS treatment options that are soon to be available.

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Competing interests
A. Methner declares associations with the following companies: Biogen Idec, Teva Pharmaceutical Industries. See the article online for full details of the relationships. F. Zipp declares no competing interests.

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BONE RESEARCH IN 2012

The ups and downs of bone in health and rheumatic disease

Ulrike Harre and Georg Schett

In 2012, several new concepts emerged that widen our view of the regulation of bone mass in health and disease. Three key studies outline these discoveries, which affect our understanding of the skeletal system, particularly its physiological function and the changes it undergoes during inflammatory disease.

Harre, U. & Schett, G. *Nat. Rev. Rheumatol.* **9**, 67–68 (2013); published online 8 January 2013; doi:10.1038/nrrheum.2012.219

Over one's lifetime, bone remodelling is tightly regulated through a complex network of hormones, cytokines and direct cellular interactions. Disturbances in this network can lead to either increased bone mass (osteopetrosis) or, much more frequently, to increased bone loss (osteopenia).[1] At the local level, bone remodelling is guided by tightly regulated crosstalk between osteoblasts and osteoclasts.[2] As bone formation and bone resorption cannot occur simultaneously at the same skeletal site, mechanisms of mutual inhibition of osteoblasts and osteoclasts have been hypothesized, to enable either bone formation or bone resorption at one specific site.

Hiroshi Takayanagi's group has now discovered the molecular mechanism of this crosstalk; that is, how bone formation by osteoblasts simultaneously blocks bone resorption by osteoclasts. Thus, semaphorin 3A, first described as an axon-guiding molecule,[3] is produced by osteoblasts and inhibits receptor activator of nuclear factor κB ligand (RANKL; also known as tumor necrosis factor ligand superfamily member 11)-induced osteoclastogenesis in bone marrow-derived monocytes.[4] The authors demonstrated that, by binding to its receptor neuropilin-1, semaphorin 3A competitively binds plexin-A1 and thereby suppresses the formation of a plexin-A1–TREM2–DAP12 complex, which provides the co-stimulatory signal needed for osteoclast differentiation. In the absence of semaphorin 3A, RANKL signalling causes rapid downregulation of neuropilin-1, resulting in the liberation of plexin-A1 and subsequent osteoclast differentiation. Semaphorin 3A thus prevents differentiation of osteoclasts by keeping monocytes in a 'stand-by' mode, which supports bone formation. Additionally, semaphorin 3A has a positive autocrine effect on osteoblast differentiation by stimulating the canonical Wnt–β-catenin pathway. In accordance with these mechanisms, mice deficient for semaphorin 3A are severely osteopenic and, conversely, treatment with semaphorin 3A blocks bone loss after ovariectomy in mice.[4] These *in vivo* data indeed suggest that semaphorin 3A has a major role in local bone homeostasis by coordinating the interplay between osteoblasts and osteoclasts. Increasing the activity of semaphorin 3A in bone could thus shift the balance of bone turnover towards formation; this approach is fundamentally different from current therapeutic intervention in osteoporosis, which reduces pathologically enhanced bone turnover in general rather than rebalancing the distorted bone turnover.

Aside from local control of bone balance, the function of bone-resorbing osteoclasts and bone-forming osteoclasts is modulated on a systemic level by the immune system.[1,5] In 2012, two entirely new interactions between the immune system and the skeleton have been discovered, which affect our understanding of rheumatic disease.

One of these findings links autoantibodies to bone loss. Autoantibodies against

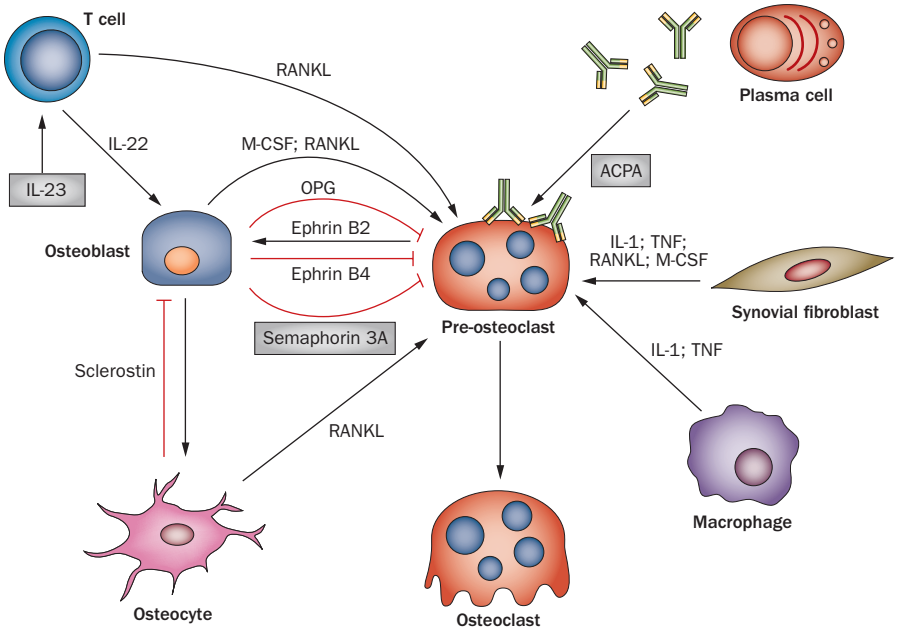

Figure 1 | Osteoimmune crosstalk regulates osteoblast and osteoclast differentiation and activity. Abbreviations: ACPA, anti-citrullinated-protein antibodies; M-CSF, macrophage colony-stimulating factor; OPG, osteoprotegerin; RANKL, receptor activator of nuclear factor κB ligand.

citrullinated proteins (ACPA) are considered the central autoimmune response in rheumatoid arthritis (RA) and are among the strongest risk factors for bone loss associated with the disease.[6] Harre and colleagues identified a direct link between ACPA and bone that explains the mechanism of bone loss in patients with RA.[7] ACPA isolated from patients with RA effectively enhance osteoclastogenesis driven by macrophage colony-stimulating factor (M-CSF) and RANKL, by binding directly to citrullinated vimentin on the surface of osteoclast precursor cells. Osteoclast-lineage cells express PAD2 (the key enzyme for protein citrullination) and, consequently, high concentrations of citrullinated vimentin, which is also expressed on the surface of osteoclasts, are available during osteoclast differentiation. In accordance with these data, patients with ACPA-positive RA show a higher rate of bone resorption as compared with those with ACPA-negative disease. In addition, challenge of lymphocyte-deficient *Rag1*$^{-/-}$ mice with ACPA leads to increased numbers of osteoclast precursor cells, enhanced osteoclastogenesis and systemic bone loss.[7] The mechanism by which ACPA boost systemic numbers of osteoclast precursor cells is based on the induction of TNF expression in these cells and the subsequent upregulation of pro-osteoclastogenic surface-bound receptors such as RANK and M-CSF receptor. These findings have linked humoral autoimmunity to bone loss, which is highly relevant for explaining the development of osteoporosis in autoimmune diseases associated with autoantibody production.

Interestingly, not only bone resorption, but also bone formation, is controlled by the immune system, as Daniel Cua's group demonstrated in a paper published in 2012.[8] They discovered that overexpression of the cytokine IL-23 induces bony proliferations along the insertion sites of tendons (entheses).[8] These bone lesions, termed osteophytes, mimic the skeletal changes observed in spondyloarthritis (SpA),[8] which is characterized by new bone formation at peripheral and axial sites and which lead to stiffness and pain.[9] Overexpression of IL-23 induced the synthesis of several inflammatory cytokines and chemokines, such as IL-6, CXC-chemokine ligand 1 and IL-22, in the inflamed entheses. In particular, IL-22 was shown to upregulate osteogenic genes, such as those encoding alkaline phosphatase, Wnt family members and bone morphogenic proteins.[8] The authors further claim a direct interaction of IL-22 with osteoblasts that leads to STAT3-phosphorylation and osteoblast activation; however, this effect was shown in an osteoblast cell line and needs to be confirmed in primary cells. The findings from Cua's group are particularly interesting because polymorphisms in the gene encoding IL-23 have been described in psoriasis and SpA. Furthermore, ustekinumab, a blocker of both IL-12 and IL-23, shows clinical efficacy in patients with psoriasis, which shows clinical overlap with SpA. The finding that IL-23 promotes new bone formation bridges these genetic associations and treatment effects with new bone formation along the entheses—a central feature of SpA.

Overall, 2012 witnessed the emergence of several new concepts regarding the regulation of bone homeostasis (summarized in Figure 1). Basic concepts of local osteoclast–osteoblast interaction have been described, as well as key mechanisms of autoantibody-mediated bone loss in RA and the mechanisms of pathological formation of new bone in SpA.

Key advances

- Semaphorin 3A, an intrinsic regulator of osteoclast differentiation, is an important regulator of bone balance[4]
- Anti-citrullinated protein antibodies directly promote osteoclast differentiation and bone loss in rheumatoid arthritis[7]
- IL-23 is a key trigger of bone-spur formation in spondyloarthritis[8]

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Competing interests
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OSTEOARTHRITIS IN 2012

Parallel evolution of OA phenotypes and therapies

Philip G. Conaghan

2012 has witnessed new developments in targeted therapies for osteoarthritis, and in ways to identify those patients who might benefit most from their application. Together, these advances could go some way to addressing the urgent need for disease-modifying treatments for this common disease.

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The growing burden of osteoarthritis (OA) in ageing societies, coupled with a paucity of effective symptomatic therapies and lack of licensed structure-modifying agents, means there is a huge unmet need for effective therapies.[1] The past 2 decades have seen major advances, led by MRI studies, in our understanding of OA pathology. The knowledge derived from improved person-specific phenotyping has started to influence

consideration of pathology-targeted therapies, and should also lead to improved application of modern therapeutic strategies (Figure 1). Several key publications from 2012 illustrate these concepts.

One of the major messages from MRI studies has been that multiple tissue pathologies are frequently seen in people with at least moderate radiographic evidence of OA[2]—the level of joint damage required for inclusion in most studies of OA symptoms or structure modification. Inflammation, especially synovitis, and bony pathology are now accepted as important in both symptom generation and possibly in structural disease progression.[2,3] Anti-TNF therapy has revolutionized the management of rheumatoid arthritis, and, given that TNF also is found in OA synovitis, its use in OA required evaluation. Researchers in Canada reported in 2012 the results of an open-label study of 20 people with painful radiographic OA knee treated with adalimumab 40 mg subcutaneously every other week (the usual dose for rheumatoid arthritis) for 12 weeks.[4] The rather strict OARSI–OMERACT response criteria were achieved by 70% of patients, and reductions in pain of 20% and 50% from baseline were reported by 70% and 40% of patients, respectively. Even allowing for the high rate of placebo response expected with this study design, these outcomes seem to be good. Half the patients still had a good response 10 weeks after treatment was discontinued, which is important when considering the putative cost-effectiveness of expensive anti-TNF therapies.

Another study of anti-TNF therapy randomized 60 patients with erosive hand OA to receive either adalimumab or placebo over 12 months.[5] Notably, the primary outcome of this trial was radiographic evidence of structural damage progression; however, the paucity of structure modification trials in hand OA means data on the responsiveness of such tools is limited. Fewer patients in the adalimumab group than in the placebo group had progression of structural damage in at least one new interphalangeal joint, but this difference was not statistically significant; in the subpopulation of patients with soft tissue swelling at baseline, however, the anti-TNF therapy did significantly reduce erosion progression. In light of another recent publication showing that ultrasonography-detected synovitis was more common in erosive hand OA,[6] we are now starting to understand how to stratify cohorts of patients with OA when employing antisynovial therapies.

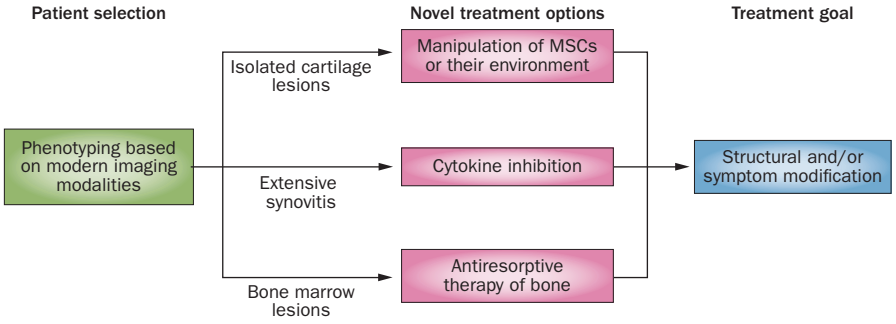

Figure 1 | Putative evolution of OA phenotypes and therapies. Modern imaging modalities have increased our understanding of the pathology of OA, enabling improved phenotyping at the level of the individual patient. In turn, patients could be selected for the treatment stream most likely to improve their condition. Coupled with the advent of novel treatment options, these advances could help achieve effective disease-modifying therapy for OA. Abbreviations: MSC, mesenchymal stem cell; OA, osteoarthritis.

Uniquely, MRI detects bone marrow lesions (BMLs), which are a common pathology of OA and are associated with pain and subsequent compartment-specific cartilage loss.[2] A 6-month, double-blind, randomized controlled trial of 59 patients with OA—selected for the presence of BMLs—demonstrated that the bisphosphonate zoledronic acid successfully reduced both pain and the size of BMLs.[7]

The insights gained from MRI have nonetheless raised new difficult questions, of which the most fundamental relates to the definition of OA. Many clinicians have a radiographic-based view of this disease, and clinical trials have often required radiographic entry criteria that employ assessment of joint-space width (a surrogate marker of cartilage loss) and marginal osteophytes. However, a new study from a community-based Boston cohort used MRI to image the knees of 710 people aged >50 years and no radiographic evidence of tibiofemoral OA.[8] An MRI abnormality suggestive of OA was present in 89% of the participants, with osteophytes, cartilage damage and BMLs (histologically associated with areas of fibrosis and trabecular remodelling) seen in 74%, 69% and 52%, respectively. About one-third of participants had painful knees (defined using a variety of patient-reported outcomes), and the prevalence of MRI abnormalities was slightly higher in these individuals (90–97%, compared with 86–88% of those without knee pain). BMI did not influence the prevalence of the MRI findings. This work has important implications for clinical trials employing radiographic definitions of OA: most extant epidemiologic and genetic studies have employed such definitions, and this may have led to misclassification and missed associations. This study also raises questions about the peripheral pathologies and other factors associated with pain. To date, pathology of the synovium and bone—both richly innervated tissues—has shown the strongest associations with pain in OA cohorts at the group level, although deciphering the complexity of peripheral nociceptive influences at the level of the individual patient remains a key area for research.

Structure modification has traditionally centred on regrowth or repair of hyaline cartilage. Given this tissue is essentially avascular, its capacity for regeneration is low. Even in OA tissue, however, mesenchymal stem cells (MSCs; plentiful in adipose tissue and bone marrow) retain regenerative capacity, and, therefore, an important field of research is based on the manipulation of MSCs or their environment to provide potential OA interventions.[9] Johnson and colleagues[10] used high-throughput screening to identify a small molecule, called kartogenin, that promotes differentiation of endogenous MSCs into chondrocytes. This molecule exhibited chondroprotective

Key advances

- An open-label study has demonstrated the successful use of anti-TNF agents as symptom-modifying therapy in OA, perhaps distinguishing key mediators in peripheral pain pathways[4]
- The limitations of radiography are evident when pathology typical of OA is demonstrated by MRI in the majority of knee joints of a large cohort aged >50 years with no radiographic OA[8]
- Manipulation of endogenous stem cells by a small molecule results in a chondroprotective phenotype both *in vitro* and in small-animal models of OA[10]

features *in vitro*; for example, in pellet culture systems, kartogenin treatment led to upregulation of tissue inhibitor of metalloproteinase I, type II collagen and aggrecan. When bovine chondrocytes and explants were cultured in the presence of typical OA-milieu cytokines, their production of nitric oxide was dramatically increased, as expected; treatment with kartogenin substantially reduced this nitric oxide release, as well as cytokine-induced release of glycosaminoglycan. Furthermore, in mouse collagenase and ligament-transection models of OA, intra-articular administration of kartogenin resulted in significant improvement of cartilage matrix and reduction in both size of cartilage lesions and circulating levels of markers of cartilage damage. Gene expression analyses suggested this molecule might work via a downstream effector, Runt-related transcription factor 1 (RUNX1).

Whilst this novel small-molecule work is truly exciting, the message from the improved understanding of the *in vivo* OA phenotype is clear: if such therapies are to be effective in human OA, they will probably need to be applied when early-stage cartilage defects are present and before substantial multitissue damage has occurred. Encouragingly, we now have the imaging tools to stratify patients for such intervention studies. This co-application of targeted molecular advances with appropriate selection of patients offers real hope for successful therapy of OA.

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RHEUMATOID ARTHRITIS IN 2012

Progress in RA genetics, pathology and therapy

Ronald F. van Vollenhoven

van Vollenhoven, R. F. *Nat. Rev. Rheumatol.* **9**, 70–72 (2013); published online 8 January 2013; doi:10.1038/nrrheum.2012.232

Developments in our knowledge of the aetiology and pathogenesis of rheumatoid arthritis continued apace in 2012, and several important new advances were reported in the therapy of this disease. Culminating in the approval of a new therapy in the USA, the year offered new insights for clinicians and fresh hope for patients.

Among many substantial advances in research in rheumatoid arthritis (RA), the top developments in 2012 touch on three different areas: genetics, osteoimmunological mechanisms of pathology, and therapy. Following an auspicious beginning in January, with the characterization of a part of the genetic basis of susceptibility to RA,[1] further progress included identification of a mechanistic link between the adaptive immune system and bone,[2] and positive trial data[3] that led to FDA approval, in November, of tofacitinib for RA. In this article I introduce these major advances and summarize other areas of promising progress in RA in 2012.

The genetics of RA have been a focus of investigation for nearly 40 years, and genetic predisposition to the disease has now been refined to the level of individual amino acids. In a large, international collaborative study, Raychaudhuri *et al.*[1] demonstrated that the increased risk of acquiring the disease that had previously been linked to the expression of certain *HLADR* genes, can be attributed to five specific amino acid locations in different HLA molecules (including not only HLA-DR, but also HLA-B and HLA-DP). Indeed, almost the entire risk of developing RA associated with the MHC could be attributed to these locations, a finding that has rejuvenated the shared epitope hypothesis. Because each of these five residues are located in the antigen-binding groove of the HLA molecule (two of them within the shared epitope)—thus, presumably, altering its antigen-binding affinity—the discovery might help in identifying putative, potentially citrullinated autoantigen(s) involved in the initiation of the disease. Besides identifying pathogenic triggers, characterization of such antigens might also result in the development of antigen-specific tolerizing regimens; such advances remain, however, a long-term prospect.

Two different ways of dividing RA into broad subtypes are well established: the first subdivision distinguishes, on the basis of radiographs, between patients with and those without erosive (destructive) disease, whereas the second concept differentiates, on the basis of serology, between those who are and those who are not positive for anticitrullinated peptide antibodies (ACPA). Approximately 70% of patients with RA express ACPA, which are associated with the presence of shared epitope alleles; indeed, the population assessed by Raychaudhuri *et al.*[1] as discussed above

was entirely ACPA positive. Now, a study by Harre *et al.*[2] has for the first time provided a mechanistic link between the ACPA-positive and erosive disease subtypes. In their *in vitro* experiments, the investigators demonstrated that osteoclasts express an epitope recognized by citrullinated vimentin-specific ACPA (one of the major types of ACPA). Furthermore, presence of these ACPA in the culture medium increases osteoclastogenesis and brings about an increase in resorption pit numbers, the *in vitro* correlate of bone damage. These findings strengthen the clinically observed link between ACPA and erosive disease, provide a mechanism for it, and suggest appropriate targets for intervention.

In RA therapeutics, the arrival of tyrosine kinase inhibitors in the clinic marks an important step forward. In November 2012, one of these agents, the Janus kinase inhibitor tofacitinib, was the first to be approved by the FDA as a second-line therapy for adults with RA. This decision followed publication of data from a large phase III clinical trial of the efficacy of tofacitinib in patients with RA and an insufficient response to methotrexate.[3] In the study, the new oral DMARD was compared not only with placebo but also with the well-established anti-TNF agent adalimumab, all with background methotrexate treatment. After 6 months, the proportion of patients who had achieved clinical responses matching American College of Rheumatology (ACR) criteria for improvement (ACR20, ACR50 and ACR70 responses) were superior for each treatment compared with placebo, and were numerically similar between the two active treatments.[3] These results, therefore, place tofacitinib at a level of clinical efficacy comparable to that of the anti-TNF agents as a class. Efficacy in terms of radiological progression, however, was not examined.

The safety of tofacitinib was studied in the comparative trial[3] as well as in a companion study of tofacitinib monotherapy (without methotrexate),[4] in addition to other studies in the development program for this drug. Overall, the safety and tolerability of this agent seem to be good, but—as is the case for many effective antirheumatic therapies—an increased risk of infection has been recognized.[3,4] Tofacitinib is also associated with potential adverse events that warrant monitoring of patients through laboratory tests; most notable among the warning signs are increased levels of transaminases and cytopenias. The clinical implications of a slight increase in creatinine level and of variable increases in levels of both HDL and LDL cholesterol, as seen with tofacitinib treatment in these trials,[3,4] are not yet clear.

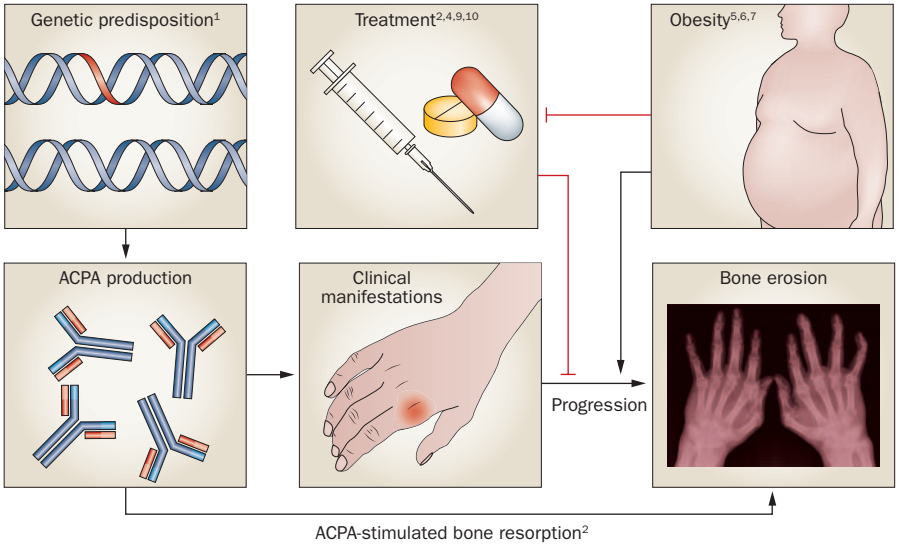

Figure 1 | Research in 2012 illuminates susceptibility to and aetiology of RA, extends treatment options, and shows that obesity modifies the effects of treatment. Raychaudhuri *et al.*[1] found that genetic predisposition to RA in MHC genes is explained by HLA modifications that alter the antigen-binding groove, potentially changing affinity for citrullinated antigens and boosting ACPA production. Harre *et al.*[2] demonstrated that ACPA stimulate osteoclast-mediated bone resorption. Clinical trial data showed tofacitinib to be safe and efficacious in RA,[3,4] and clarified the effects of DMARD combinations.[9,10] Obesity is associated with worse outcomes in RA,[5,6] and the efficacy of anti-TNF therapy was shown to be blunted in the presence of obesity.[7] Abbreviations: ACPA, anticitrullinated protein antibodies; RA, rheumatoid arthritis.

Many other important developments in RA have taken place in 2012; more than editorial limits permit me to name. Among other notable findings, the relationship between obesity and RA was studied by several investigators. Both Ajeganova *et al.*[5] and Wolfe and Michaud[6] showed that obesity is associated with worse clinical outcomes in patients with RA. Furthermore, Stavropoulos-Kalinoglou *et al.*[7] showed that whereas anti-TNF therapy improves insulin sensitivity in normal-weight individuals with RA, the same is not true in overweight patients. These findings emphasize that maintenance of a healthy weight should form an important objective for patients with RA.

Tofacitinib does not represent the only advance in RA therapeutics in 2012. Publication of 3-month data from 281 patients in the tREACH trial confirmed earlier findings showing that combination DMARD therapy (including methotrexate) is superior to methotrexate monotherapy in early RA.[8] The DMARD treatments, including methotrexate alone, were combined with glucocorticoids as bridging therapy; an oral, tapered glucocorticoid regimen over 16 weeks and a one-off intramuscular dose alongside the combination DMARDs were both superior to glucocorticoids plus methotrexate.[8] Meanwhile, Moreland *et al.*[9] demonstrated in the TEAR trial that combination therapy with conventional DMARDs in early RA achieved similar clinical and radiographic results to those of combined methotrexate and etanercept, whereas 2-year follow-up data from the SWEFOT trial upheld only a small radiographic benefit (and no significant clinical benefit) for the combination of methotrexate with infliximab, versus a nonbiological DMARD regimen.[10] Thus, clinical trials continue to support early aggressive treatment of RA

Key advances

- Five amino acids in the antigen-binding groove of HLA molecules account for most of the association between MHC genes and susceptibility to rheumatoid arthritis (RA)[1]
- Anticitrullinated protein antibodies (ACPA) stimulate osteoclastogenesis *in vitro*, mechanistically linking the clinically associated erosive and ACPA-positive subtypes of RA[2]
- Phase III data demonstrate the efficacy and safety of the Janus kinase inhibitor tofacitinib in RA[3]

using conventional agents, whereas the role of biologic agents as first-line therapy remains incompletely defined.

All in all, 2012 was an excellent year for RA research (Figure 1), but many questions remain. Major topics for ongoing research are: the identification of environmental triggers in the susceptible host; the site of initial inflammation; the possibility of 'induction–maintenance' therapy in both early and established RA; and the emerging role for tyrosine kinase inhibitors in the treatment of this disease. It will be interesting to find out which of these issues can be resolved in 2013!

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SPONDYLOARTHRITIS IN 2012

Advances in pathogenesis through animal models and imaging

Walter P. Maksymowych

Advances in 2012 have helped to solve several established mysteries in spondyloarthritis (SpA)—why T-cell-directed therapies have not delivered the expected efficacy and how the IL-23–IL-17 cytokine axis is linked to the specific pathology of SpA. The opportunity to influence disease progression at inflammatory lesions using anti-TNF agents may be fleeting.

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Spondyloarthritis (SpA) studies in the past few years have demonstrated a puzzling lack of an effect of anti-TNF therapy on new bone formation as assessed by radiography, despite substantial benefits for symptoms, and a surprising lack of efficacy for the T-cell-directed agent, abatacept.[1] Meanwhile, in a preliminary study, an anti-IL-17 agent was shown to have a clinical benefit and SpA was consistently shown to be associated with polymorphisms in the IL-23 receptor (*IL23R*) gene locus,[2] which altogether reinforce the growing importance of the IL-23–IL-17 cytokine axis in the pathogenesis of SpA. However, investigators have so far been unable to show an association of cytokine biology with the defining clinical characteristics of the disease, especially those of enthesitis.[2] Several studies in 2012 have provided important insights into each of these issues and defined priorities for future research.

A major clinical challenge in human SpA is the lack of objective manifestations; thus, the disease is often at an advanced stage by the time patients present clinically, and particularly when they are recruited to clinical trials. The generally slow evolution of radiographic changes of SpA precludes randomized study designs with comparator groups, and a minimum study duration of 2 years is required to achieve reliable detection of radiographic progression; these factors pose limitations for the conclusions drawn from anti-TNF trials. Direct non-invasive assessment of spinal inflammatory lesions over time is possible using MRI, and previous work using both short-tau inversion recovery (STIR) and T1-weighted (T1W) sequences has shown that resolving inflammatory lesions undergo fat metaplasia (detected on T1W MRI), and that this feature is frequently observed in the majority of patients with SpA. Consequently, assessment of the link between inflammation and new bone formation using MRI requires assessment of both STIR and T1W sequences, although previous reports have focused only on STIR.

In a 24-week placebo-controlled trial of the TNF inhibitor adalimumab, with 2-year extended open-label observation, Maksymowych *et al.*[3] assessed the association between inflammation at vertebral corners on baseline MRI and newly formed syndesmophytes on 2-year radiographs in 76 patients. Simple inflammatory lesions that were visible only on the STIR sequence resolved completely after adalimumab administration without any sequelae.[3] Whereas inflammation in more complex lesions associated with fat metaplasia on T1W MRI also resolved, this finding was associated with new syndesmophytes which were evident even after correcting for the extent of radiographic changes at baseline, a known predictor of future progression. New syndesmophytes developed at the site of complex lesions irrespective of whether inflammation persisted or resolved. The majority of new syndesmophytes occurred at vertebral corners that had either an inflammatory and/or fat lesion.

The major implications of this study[3] are twofold: first, whereas inflammation is linked to new bone formation through a process of fat metaplasia, eventually, these two processes diverge and bone formation becomes autonomous. The failure to demonstrate an effect of anti-TNF agents in SpA might thus be due to most patients involved in trials having both simple inflammatory and complex lesions. Therefore, anti-TNF therapy would have no net effect on new bone formation over 2 years—benefit would be observed only after longer follow-up, as prolonged treatment prevents development of new inflammatory lesions. Second, the

Key advances

- MRI indicates an opportunity for disease modification using anti-TNF therapy[3]
- A new mouse model of SpA demonstrates the diversity of IL-23 signalling pathways associated with development of the disease[8]
- The IL-23-overexpressing mouse model highlights the role of IL-23 in entheseal inflammation characteristic of SpA[10]

data are in support of early intervention with anti-TNF agents when spinal inflammation is found on MRI, and testing of this hypothesis constitutes a high priority for future research.

Despite reports of lymphocytic infiltrates at sites of inflammation in SpA, the physiological role of HLA-B27 in presentation of antigens to $CD8^+$ T cells, and increased numbers of peripheral T helper cells expressing IL-17 (T_H17 cells) reported in patients with SpA,[4] intervention with abatacept has failed to produce any clinical benefit. This result, as well as recent reports showing that mast cells,[5] γ/δ T cells,[6] and cells of myeloid lineage[7] are the primary cell types expressing IL-17 at sites of inflammation in SpA, have cast doubt on the primary role of adaptive immunity and have drawn attention to innate immune pathways as the primary basis for inflammation in SpA.

Ruutu *et al.*[8] have described a new animal model of SpA, in which autoimmune-prone SKG mice carrying a Zap-70 mutant that increases the autoreactivity of T cells develop peripheral and axial arthritis (including sacroiliitis, erosive disc lesions, facet joint inflammation, osteophytes, enthesitis, and dactylitis) following administration of curdlan, which comprises β-glucan aggregates and is commonly found in fungal and bacterial cell walls. Extra-articular features of this SpA model resembled those of human SpA and included iritis and ileitis (histologically resembling Crohn's disease), but not colitis or typical psoriasis.

The proinflammatory innate effects of β-glucan are mediated through signalling via its receptor dectin-1, which promotes expression of IL-1β, 1L-12, and IL-23. IL-23 is required for expansion of T_H17 cells, and inhibition of IL-23 in the SKG mice prevented the development of arthritis but not ileitis. The pathology was transferable to SCID mice by $CD4^+$ T cells and was associated with expression of type II collagen-specific and proteoglycan-specific antibodies. Administration of an additional cell wall component, mannan, also triggered disease, demonstrating the importance of interaction between the innate and adaptive arms of the immune system in the development of an IL-23-dependent, tissue-specific, autoimmune response.

Although other animal models of SpA are T-cell independent,[9] the β-glucan SKG model and the polygenic basis of human SpA highlight the importance of several potential signalling pathways leading to inflammation that are likely to depend on genetic background. This concept has important implications for prediction of therapeutic response and the design of clinical trials.

Although tissue-specific autoimmune inflammation might be dependent on activation and expansion of autoreactive T cells, this mechanism has been difficult to demonstrate in human SpA. Sherlock *et al.*[10] hypothesized that entheses themselves contain IL-23-responsive T cells that are capable of initiating inflammation. In IL-23R-eGFP reporter mice, they performed flow cytometry analysis of $CD45^+$ entheseal cells and showed that the $IL\text{-}23R^+$ cell fraction expressed CD3, but not CD4 and CD8. Using multiphoton microscopy on live, unmanipulated tissue, they showed that $IL\text{-}23R^+$ T cells were located at the entheseal interface between the tendon and the bone of both axial and peripheral entheses, and were absent from the tendon proper.[10] Entheses in culture responded to IL-23 through upregulation of the expression of IL-17, IL-22, and bone morphogenetic protein-7. Overexpression of IL-23 using minicircle DNA technology in the hepatocytes of mice was sufficient to lead to severe entheseal inflammation with infiltration by macrophages and neutrophils, and expansion of periosteal osteoblasts. Disease features included sacroiliitis, axial enthesitis, psoriasis, and aortic root inflammation. Upregulation of bone remodelling genes such as *Runx2* and *Sp7* was not evident until inflammation had been present for several weeks. The disease was not ameliorated by neutralization of TNF or IL-6.[10]

Further characterization of the $CD3^+$ T-cell population demonstrated expression of the key transcription factor ROR-γ in IL-23-responsive T cells as well as IL-17 and IL-22, but not of surface markers indicative of γ/δ T cells or NK cells.[10] Depletion of T_H17 or $CD4^+$ T cells did not inhibit the course of the disease, but neutralization of IL-17 or IL-22 with antibodies was effective, particularly when both antibodies were administered together. Notably, overexpression of IL-22, but not IL-17, using minicircle

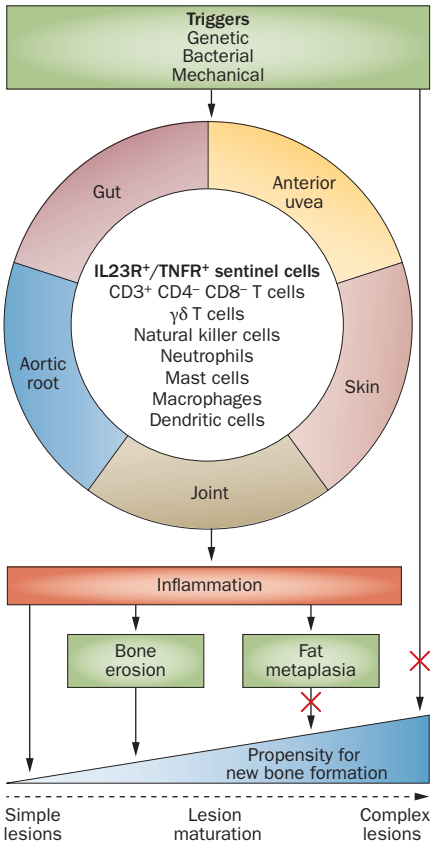

Figure 1 | Lesion formation in SpA. The tissue-specific inflammatory hyper-reactivity to aetiological stimuli in SpA is mediated by a diversity of tissue-localized $IL\text{-}23R^+$ and $TNFR^+$ sentinel cells with roles in innate immunity. Inflammation triggers reparative pathways which operate in an increasingly autonomous manner and become unresponsive to anti-inflammatory therapies with lesion maturation. Complex lesions with fat metaplasia and new bone formation involving noninflammatory pathways might not be amenable to disease modification by anti-TNF agents (denoted by red crosses).

technology also led to SpA development, and IL-22 was more effective than IL-23 in inducing expression of genes that regulate bone formation, such as those encoding Wnt family members, bone morphogenetic proteins, and alkaline phosphatase.[10]

From these studies, SpA emerges as a disease characterized by a state of 'genetically-primed' hyper-responsiveness to specific cytokines, particularly IL-23 and TNF, through an array of $IL\text{-}23R^+$ and $TNF\ receptor^+$ sentinel cells located in certain tissues, with the source of inflammation determined by genetic, mechanical, bacterial and other triggers (Figure 1). Animal models of SpA have failed to inform us about the complexity of reparative pathways that follow inflammation in humans, but MRI data

indicate that the window of opportunity for effective disease modification using anti-inflammatory therapies could be limited.

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REGENERATIVE MEDICINE IN 2012

The coming of age of musculoskeletal tissue engineering

Rocky S. Tuan

Tissue engineering and regenerative medicine have advanced rapidly towards the development of therapeutic solutions for musculoskeletal disorders. In 2012, breakthroughs have been made in the guidance of adult stem cell homing, the tissue regenerative activity of stem-cell-derived extracellular matrix has been tested, and novel, mechanically superior biomaterials have been fabricated.

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More than two decades ago, the field of tissue engineering, built on a combinatorial platform of cells, scaffolds, and biofactors, was conceptualized as a means to functional restoration and regeneration of diseased or damaged tissues and organs, including those of the musculoskeletal system. More recently, a number of new concepts have been developed that promise to enhance the success of cell-based and biomaterial-based tissue engineering approaches. In 2012, a number of studies exemplified these new concepts and represent key advances in the approach to regenerative medicine.

Adult mesenchymal stem cells (MSCs) represent the most widely investigated cell type for musculoskeletal tissue engineering;[1] the concept that these cells have immunoregulatory, anti-inflammatory, pro-survival, and endothelial cell modulatory activities is increasingly considered in regenerative applications, in addition to their multilineage differentiation potential.[2] In order to exert these activities in a way that will influence tissue regeneration, MSCs must first home to the target tissue and subsequently differentiate appropriately.

In a 2012 study, Guan *et al.*[3] developed a conjugate molecule comprising LLP2A, a peptidomimetic ligand for α4β1 integrin receptors that were found to be highly expressed on osteoprogenitor MSCs, and the bisphosphonate alendronate, which has a high affinity for bone. *In vitro* analyses showed that this LLP2A–alendronate conjugate (LLP2A–Ale) increased MSC migration and osteogenesis.[3] *In vivo*, intravenously xenotransplanted human MSCs (hMSCs) were detected adjacent to the periosteal, endocortical and trabecular bone surfaces in the lumbar vertebral bodies of severe combined immunodeficiency mice only after co-injection with LLP2A–Ale.[3] After 3 weeks follow-up, the hMSCs were seen embedded within the bone matrix and were associated with increased bone formation.[3] Interestingly, injection of LLP2A–Ale alone also augmented bone formation;[3] the authors suggested that the 'bone-seeking' alendronate-conjugated α4β1 integrin ligand could promote homing of endogenous MSCs to bone, because LLP2A alone —which would have a nonspecific tissue distribution—was not osteogenic.[3] More importantly, injection of LLP2A–Ale alone was found to prevent trabecular bone loss in immunocompetent mice with ovariectomy-induced osteopenia, resulting in increased osteoblast and mineralizing surfaces, as well as higher rate of bone formation for the total bone surface.[3] These exciting findings demonstrate a potentially effective means of guiding MSCs to bone (Figure 1a), and provide strong evidence that MSCs can act as functional osteoprogenitor cells *in vivo*. Moreover, the effects of LLP2A–Ale alone in osteopenic mice suggest that endogenous MSCs could, in fact, be adequately recruited to bone surfaces using this molecule. Studies to test the effectiveness of this approach for the treatment of bone abnormalities, such as fractures and osteoporosis, are certainly warranted. This model should also be useful to examine whether the regulatory activities of recruited MSCs also influence cells resident in the bone to cooperatively enhance bone formation.

Tissue matrices have been recognized as rich depots of molecular signals, primarily growth factors sequestered by macromolecular components of the extracellular matrix (ECM), which regulate tissue-specific biological activities.[4] Indeed, when used as scaffolds for seeded MSCs, hMSC-derived ECM preparations, obtained by decellularizing *in vitro* MSC cultures, promote cell attachment, spreading, migration, proliferation, and maintenance of response to differentiation signals.[5] This concept formed the basis of a different approach to optimize delivery and maintenance of MSCs that was developed in a 2012 study

by Zeitouni *et al.*[6] Injection of hMSCs, pretreated with GW9662 to suppress adipogenic activity via inhibition of peroxisome proliferator-activated receptory, improved bone healing in a mouse calvarial defect xenograft model;[6] however, the effect was limited to the early osteogenic phase, and was lost during bone remodelling due to unsatisfactory hMSC retention.[6] 3 weeks after grafting of GW9662-treated hMSCs together with hMSC-derived ECM, increased hMSC engraftment was detected, compared with mice treated with hMSCs alone, and reproducible, near-complete repair of calvarial bone defects was seen (Figure 1b),[6] indicating that the hMSC-derived ECM acted as a retentive scaffold. In mice treated with hMSC and hMSC-derived ECM, large clusters of engrafted cells were observed that were not associated with the remodelling bone surface,[6] suggesting that the matrix-localized hMSCs provided an enabling microenvironment that promoted intrinsic bone formation within the graft. These findings strongly suggest that MSC-derived ECM should be considered as a potential adjunct component in the design of bioactive biomaterial scaffolds for tissue engineering.

Researchers are increasingly realizing that tissue or organ regeneration must recapitulate the biological mechanisms of embryonic development and tissue morphogenesis,[7] not only at a molecular level, but also in terms of the spatiotemporal and physical aspects.[8] This understanding has led to increasingly rational design of biomaterial scaffolds, the development of 3D tissue constructs and their propagation in bioreactors that provide more native environments. Because of the weight-bearing mechanical requirements of musculoskeletal tissues, a preferred bioactive scaffold would consist of a mechanically strong biomaterial scaffold, such as a bioresorbable polymer, that appropriately incorporates decellularized ECM. Hydrogels are popular biomaterials for tissue engineering, drug delivery and modelling of ECM owing to ease of manipulation and cell seeding prior to gelation, but are severely limited by their mechanical behaviour, particularly their low stretchability.

Sun *et al.*[9] recently reported a new protocol for synthesis of highly stretchable and tough hydrogels. Gelation of hydrogels is normally mediated via either ionic cross-links, such as in alginate, or covalent cross-links, such as in polyacrylamide. Sun and co-workers[9] created an alginate–polyacrylamide hybrid hydrogel, in which the two types of polymer network

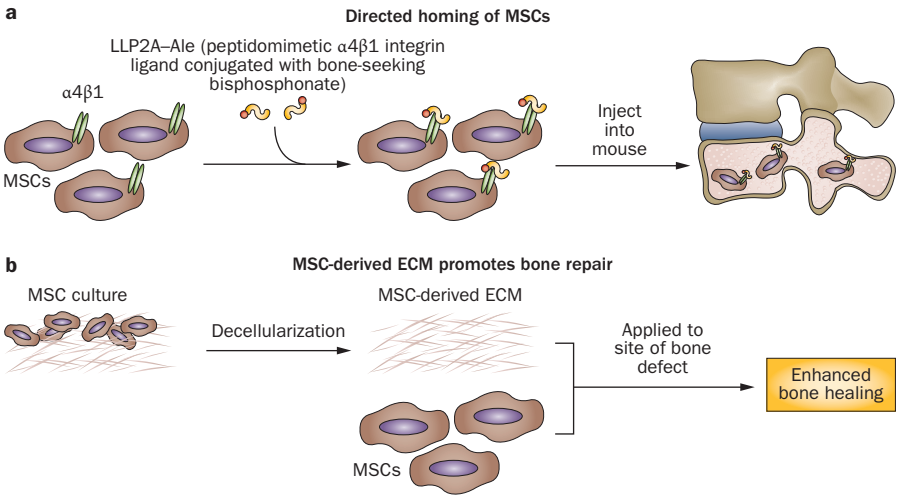

Figure 1 | New approaches to enhance MSC-mediated tissue repair. **a** | Guided homing of MSCs to bone surface using an integrin-binding peptidomimetic ligand, LLP2A, conjugated with the 'bone-seeking' bisphosphonate alendronate (LLP2A–Ale). **b** | MSC-derived ECM co-administered with MSCs to enhance MSC retention and healing of bone defects. Abbreviations: ECM, extracellular matrix; MSC, mesenchymal stem cell.

were intertwined and further cross-linked via covalent bonds between amine groups on polyacrylamide chains and carboxyl groups on alginate chains. The hybrid gels contained ~90% water, but could be stretched beyond 20 times their original length, and had high fracture energies of around 9,000 J/m^2 compared with 10–250 J/m^2 for alginate or polyacrylamide gels;[9] even when notches were created in the hydrogel structure, the stretchability was up to 17 times the original length.[9] The authors attributed the large improvement in hydrogel mechanical properties to two factors: crack bridging by covalent cross-links and the network of ionic cross-links providing hysteresis. A nascent engineered or regenerated tissue initially produced by seeded cells is unlikely to exhibit, on its own, the physical properties of the mature tissue; thus, the biomaterial scaffold has the burden of meeting the physicomechanical requirements. In this respect, the principle of combining weak and strong cross-links between brittle constituents to produce a tough, stretchable material should be of high utility. Such an approach should minimize physical damage of the nascent engineered tissue construct and enhance its eventual maturation and integration into the host tissue.

Although ionic cross-links, mediated by divalent cations for example, are generally biocompatible, free-radicals produced during chemical covalent cross-linking are cytotoxic and the reaction can also be exothermic. Photo-induced covalent cross-linking might represent a milder alternative,[10] but

Key advances

- Bisphosphonate-directed, integrin-dependent homing of mesenchymal stem cells (MSCs) to bone improved bone formation and retention in mice[3]
- MSC-derived extracellular matrix has been used successfully as a bioactive scaffold to enhance bone repair *in vivo*[6]
- Ionic and covalent cross-links between mechanically weak components improve hydrogel stretchability and stiffness[9]

is generally slower than the chemical cross-linking method. To be adopted for cell-based tissue engineering, these issues will need to be resolved to make hybrid hydrogels biocompatible, enabling their evaluation in direct cell-seeding protocols.

Tissue engineering and regenerative medicine are highly active research disciplines that have seen rapid, complementary convergence of life science research, engineering, and clinical medicine, illustrated by the advances in MSC grafting and biomechanical scaffold design made in 2012 and described herein. The translational nature of this discipline dictates that issues such as biocompatibility, scale-up and safety must be considered, to enable preclinical and clinical trials that will lead to therapeutic product development for musculoskeletal repair and regeneration.

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Competing interests
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MICRORNA IN 2012

Biotherapeutic potential of microRNAs in rheumatic diseases

Yves-Marie Pers and Christian Jorgensen

A number of microRNAs have been implicated in the pathogenesis of various rheumatic diseases, and evidence in support of the therapeutic potential of microRNA-based strategies for these conditions is growing, as demonstrated by several new findings published in 2012.

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MicroRNAs (miRNAs) are small noncoding RNA molecules that modulate the expression of multiple protein-encoding genes at the post-transcriptional level.[1] These molecules participate in nearly every developmental and physiologic process. Although the function of most mammalian miRNAs has yet to be determined, it seems that their aberrant expression could have a role in the pathogenesis of several osteoarticular diseases.[1] Several important insights in 2012 have implicated specific miRNAs in the pathophysiology of osteoarthritis (OA)[2] and in TNF-driven[3] and nuclear factor κB (NFκB)-driven[4] inflammation. These miRNAs are involved in chondrogenic differentiation pathways as well as in the immune response, and are putative therapeutic targets.

Key advances

- miR-194 is reportedly involved in the pathophysiology of osteoarthritis and potentially has a role in induction of chondrogenesis[2]
- miR-323-3p is overexpressed in synovial fibroblasts both from patients with rheumatoid arthritis and from transgenic mice with TNF-driven arthritis, and might regulate the Wnt–cadherin pathway[3]
- miR-23b is a central regulator of inflammation in resident tissue cells during autoimmunity[4]

miRNAs are naturally produced by cells.[1] They are derived from primary miRNA transcripts that are processed in the nucleus to form precursor miRNA (pre-miRNA), a hairpin loop of about 70 nucleotides. The pre-miRNAs are then transported to the cytoplasm where they are cleaved to produce a miRNA–miRNA* duplex of 20–22 nucleotides in length. This duplex is unwound by a helicase, and the miRNA 'guide' strand is then incorporated into the multiprotein RNA-induced silencing complex (RISC) to function as a so-called mature miRNA, whilst the 'passenger' strand (miRNA*) is released and degraded. Mature miRNAs participate in gene silencing by regulating the stability or translational efficiency of target messenger RNA (mRNA). Depending on the degree of complementarity in base-pairing between the miRNA and the target mRNA, the miRNA either represses translation (through imperfect pairing) or cleaves the mRNA (through perfect pairing).

miRNAs have been shown to have a role in maintaining cartilage homeostasis during development, and also to be dysregulated in OA. No efficient structure-modifying therapy is available for OA, a disease that results from cartilage degeneration, chondrocyte hypertrophy and synovitis. miRNA profiling in human cartilage has identified miRNA targets with relevance to. Expression of miR-140, for example, is decreased in OA cartilage in comparison with normal human cartilage, and *miR140* knockout mice develop OA-like pathology with age.[5] By contrast, miR-146a is strongly induced in IL-1β-stimulated OA chondrocytes,[6] and miR-199* induces chondrogenesis through targeting of SMAD1 and cyclooxygenase-2 in human chondrocytes.[7] Multipotent mesenchymal stem cells, in particular adipose-derived stem cells (ADSCs), have been shown to be chondroprotective through anti-inflammatory and antifibrotic properties, to protect cells from oxidative stress, to prevent senescence, and to stimulate proliferation and differentiation of chondrocytes in co-culture through the release of growth factors.[8] These findings have opened the way for novel therapeutic applications combining stromal cells and miRNA delivery for the treatment of various disorders, including OA. In their 2012 paper, Jun Xu and colleagues[2] showed that levels of miR-194 decreased during chondrogenic differentiation of human ADSCs. The down-regulation of miR-194 increased expression of SOX5 (a key transcription factor for cartilage differentiation) at protein level and resulted in enhanced chondrogenic differentiation of human ADSCs (Figure 1). The researchers also found that miR-194 was upregulated in IL-1β-induced OA, and was associated with a decrease in SOX5 expression. These data suggest that miRNAs are involved in OA pathophysiology and potentially have a role in induction of chondrogenesis.

Rheumatoid arthritis (RA), an inflammatory autoimmune disorder related to chronic synovitis, is a multifactorial disease. The identification of a key causal role for miRNAs in chronic inflammation and the pathogenesis of RA might lead to the development of a novel therapeutic strategy. Many studies have demonstrated the upregulation of several miRNAs either in

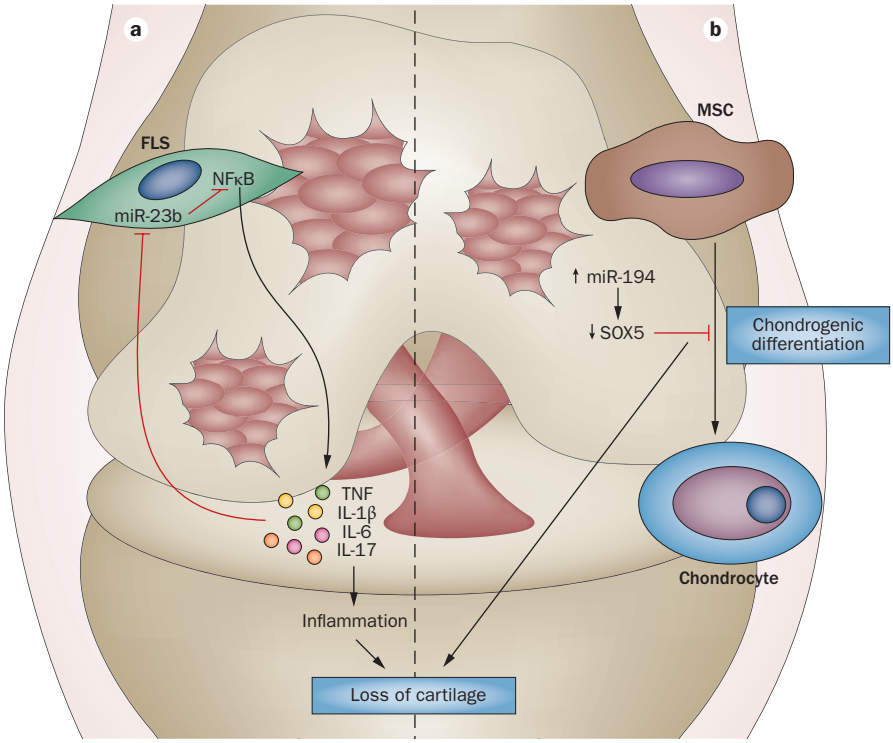

Figure 1 | New miRNA-mediated mechanisms implicated in the pathology of rheumatic diseases. Not depicted in this figure, but also discovered in 2012, is a role for miR-323-3p, which is overexpressed in FLS, modulates Wnt–cadherin signalling and could represent a therapeutic target for rheumatoid arthritis.[3] **a** | Expression of miR-23b inhibits inflammation by reducing NFκB pathway activation, but is downregulated in cells from patients with autoimmune diseases. IL-17 is thought to be a key mediator in suppressing miR-23b, but whether the reduced expression is driven by type 17 T helper cells is unknown.[4] **b** | Levels of miR-194 are decreased during chondrogenic differentiation of human MSCs, in particular adipocyte-derived stem cells. Downregulation of miR-194 increases expression of SOX5, a key transcription factor for cartilage differentiation. miR-194 is upregulated in IL-1β-induced experimental osteoarthritis, and is associated with a decrease in SOX5 expression.[2] Abbreviations: FLS, fibroblast-like synoviocyte; miRNA, microRNA; MSC, mesenchymal stem cell; NFκB, nuclear factor κB.

the circulation or within the inflamed joints of patients with RA, including miR-16, miR-132, miR-146a and miR-155.[8] Not surprisingly, among the first of these miRNAs to be investigated in RA samples were miR-146a and miR-155, both of which are involved in the development of innate and adaptive immune cells, are upregulated under inflammatory conditions and in response to a variety of microbial components,[10] and are overexpressed in several immune-mediated inflammatory disorders.[9] However, studies using a mouse model of TNF-driven arthritis have now identified novel miRNAs associated with RA, paving the way for innovative biotherapies. In 2012, Pandis *et al.*[3] studied miRNA expression in synovial fibroblast cells both in human RA and in a TNF-transgenic mouse model of the disease (TghuTNF) in which mice develop spontaneous arthritis mediated by the direct activation of TNF receptor 1 on synovial fibroblasts. They found that miR-323-3p, miR-155, miR-221 and miR-222 are overexpressed in synovial fibroblasts both from patients with RA and from TghuTNF mice, whereas miR-146 is upregulated only in the mouse model. Moreover, Pandis *et al.*[3] showed that miR-323-3p enhances activation of the Wnt–cadherin pathway in human RA synovial fibroblasts by targeting β-transducin repeat-containing protein, a negative regulator of β-catenin, suggesting that inhibition of miR-323-3p could be a therapeutic strategy for RA.

Several miRNAs have been shown to be deregulating in autoimmune diseases and chronic inflammatory disorders. A key paper published in 2012 by Shu Zhu *et al.*[4] provides strong evidence in support of a role for another miRNA in autoimmune diseases: miR-23b. The investigators showed that miR-23b is downregulated in RA, systemic lupus erythematosus and multiple sclerosis, both in human resident tissue cells and in different mouse models of these diseases. miR-23b strongly inhibits inflammation by reducing activation of the NFκB pathway, leading to the dramatic decrease in expression of inflammatory cytokines such as TNF, IL-1β and IL-17. Moreover, overexpression of miR-23b suppresses autoimmune pathogenesis in mouse models of RA, lupus and multiple sclerosis by inhibiting the expression of inflammatory cytokines. In autoimmune diseases, compared with other inflammatory cytokines (TNF, IL-1β or IL-6), IL-17 seems to be the key cytokine responsible for the downregulation of miR-23b expression. These data suggest a central role of miR-23b in autoimmunity whereby IL-17 decreases miR-23b expression by resident tissue cells, enabling activation of the NFκB pathway and potentially leading to tissue damage (Figure 1). Whether or not the downregulation of miR-23b is a consequence of a process driven by type 17 T helper cells, or if it is inherited and present in early-stage RA, remains to be determined.

The therapeutic potential of miRNAs for rheumatic diseases is promising, and we can expect rapid progress toward strong proof-of-concept studies in preclinical models for the use of miRNA-based therapeutic strategies.[1] Although not specific for RA, miR-16, miR-146a, miR-155 and miR-23b have been a particular focus for investigations into this disease as they are induced by inflammatory conditions, overexpressed in RA tissues, and have broad regulatory roles in the immune response and inflammation. Importantly, their critical roles in haematopoietic differentiation and function, and more specifically in myelopoiesis, also have consequences for osteoclastogenesis. Moreover, miR-323-3p, miR-221 and miR-222 are induced by TNF stimuli, are detected in synovial fibroblasts or others tissues, and have multiple targets representing a link between innate and adaptive immunity and bone homeostasis. More work remains to be done, but miRNAs represent a track of research that will provide new insight into the pathogenesis of chronic inflammation and autoimmune diseases, which could lead, in turn, to the development of miRNA-based therapeutics.

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Competing interests
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BLADDER CANCER IN 2012

Challenging current paradigms

Aidan P. Noon and James W. F. Catto

2012 has been a promising year for patients with bladder cancer. Published reports have challenged current knowledge in areas ranging from disease aetiology to second-line chemotherapy. However, advances in the care of this cancer lag behind those in many other malignancies, reflecting the low priority of bladder cancer to many research funders.

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The demographics of bladder cancer are changing. The disease, which has historically been prevalent in industrial nations, is becoming less common in the western world and more common in developing countries. This change reflects a decline in cigarette smoking and improvements in workplace hygiene in many western nations, coupled with the reverse of these trends and exposure to polluted or contaminated water sources in developing nations. For example, the WHO has identified rising rates of cigarette smoking in Africa, the Middle East, Eastern Europe, the former Soviet Union and parts of Asia. Examples of pollution are becoming common, such as arsenic exposure reported in Chile this year.[1] The decline in bladder cancer diagnoses in the West is likely to continue as occult or unknown bladder carcinogens are identified and their use replaced or restricted.

In 2003, the oral hypoglycaemic drug pioglitazone (a thiazolidinedione), an agonist of the peroxisome-proliferator-activated receptor, was identified as a potential urothelial carcinogen. In 2012, further evidence of such an effect was published. A retrospective cohort study[2] found an increased risk (HR 3.25) of bladder cancer in type 2 diabetic patients exposed to thiazolidinediones ($n = 18{,}459$) for more than 5 years compared with patients taking sulphonylureas ($n = 41{,}396$). Given the possible association of bladder cancer in diabetic patients taking pioglitazone, it would seem sensible for urologists to try to identify bladder cancer patients taking this medication and discuss alternative therapies with their diabetology colleagues. In the longer term, the use of thiazolidinediones must be questioned.

For more than 30 years, the standard initial approach to treating a new or recurrent bladder tumour has been transurethral resection followed by adjuvant intravesical chemotherapy (typically, mitomycin C [MMC]). This paradigm was challenged in late 2011 by a randomized controlled trial (RCT) that compared this standard protocol with the addition of preoperative intravesical chemotherapy.[3] Di Stasi *et al.*[3] reported that electromotive-assisted instillation of MMC immediately prior to transurethral resection reduced subsequent tumour recurrence when compared with postoperative MMC or placebo (recurrence rate 38% versus 59% versus 64%, respectively ($P < 0.0001$) at a median follow-up duration of 86 months).

An alternative preoperative MMC regimen was also explored by Colombo *et al.*[4] in 2012, in a study comparing six preoperative MMC installations delivered either weekly for 6 weeks or over 2 weeks (intensive arm) before resection in patients with single small (<1.5 cm) recurrent tumours. The intensive regimen was more effective than the weekly schedule (with a remission rate of 70% versus 44.4%, respectively; $P = 0.04$) but marginally less well tolerated: two of 27 patients in the intensive arm did not complete the treatment owing to severe cystitis symptoms compared with three patients in the weekly group that had to have their treatment delayed by 1 week owing to cystitis (all patients completed the full course of six instillations). Further evaluation of this intensive treatment regimen is currently under investigation in several RCTs and could potentially obviate the need for transurethral resection in patients who are unfit for surgery or those with indolent disease.

Key advances

- Use of thiazolidinedione hypoglycaemic medication pioglitazone is shown to be a risk factor for bladder cancer[2]
- Neoadjuvant intravesical chemotherapy with mitomycin C before transurethral resection is identified as an effective approach to reducing postoperative recurrence with low associated toxicity[3]
- Chemoradiotherapy is suggested as a viable alternative to radical cystectomy for muscle-invasive disease[7]
- Mutations in the *TSC1* gene are shown to be a marker that can identify patients whose advanced tumours will respond to everolimus[10]

For many patients and physicians, 2012 will be remembered as the year of limited BCG supply. This shortage arose when a failure in the sprinkler system at Sanofi Pasteur's Canadian factory prompted an overhaul of the facility and a cessation in Immucyst® (Sanofi Pasteur, Lyon, France) manufacture. The manufacture of an alternative product, OncoTice®, (Merck Sharp & Dohme, Herfordshire, UK), struggled to meet the increased demand, owing to the latency of bacterial growth necessary for BCG production. This shortage affected patients in Europe, North America, Australia and India, amongst others. In the absence of bladder-sparing alternatives, the use of cystectomy will have increased in these regions.

Regardless of BCG supply, the unpredictable natural history of high-risk non-muscle-invasive bladder cancer makes care of these patients problematic. In 2012, van Rhijn *et al.*[5] proposed a new classification of pT1 substaging that aimed to improve the risk stratification of progression to invasion in individual tumours. The proposed classification divided T1 disease into two subgroups: T1m (microinvasive) and T1e (extensive; multiple foci or >0.5 mm lamina

propria invasion). The risk of progression and disease-specific death in patients with pT1e tumors is threefold that of those with pT1m disease. This classification seems simple to implement and could prove useful in guiding patient care until biomarker studies identify better predictive factors.

2012 also saw the failure of yet another competitor of BCG in these patients. Lammers *et al.*[6] reported the results of a phase III RCT comparing keyhole limpet haemocyanin (KLH) to MMC. Despite promising phase II data, KLH was inferior in preventing recurrence and, the difference in progression failed to reach significance when compared to MMC. However, the low progression rate in this report suggests that a longer follow-up duration might alter the interpretation of this RCT and, therefore, support the use of KLH as a viable alternative to BCG.

For patients with invasive bladder cancer, the 2012 results of a multicentre prospective phase III RCT (BC2001) comparing radiotherapy with chemoradiotherapy (5-fluorouracil and MMC) in nonmetastatic disease were reported.[7] At analysis (69.9 months median follow-up duration) a significant reduction in locoregional recurrence was observed for patients receiving chemoradiotherapy (54% versus 67% for radiotherapy alone, HR 0.68 (95% CI 0.48–0.96, $P=0.03$). This difference did not translate to disease-specific and overall survival, but was apparent in the rate of distant metastases (HR 0.72, 95% CI 0.53–0.99, $P=0.04$). Although the authors did not compare their findings to radical cystectomy, this trial defines chemoradiotherapy as a viable therapy for patients with invasive tumours. As suggested by the authors, chemoradiotherapy could be a less morbid procedure than radical cystectomy in patients with significant comorbidity or poor fitness for surgery, and still enables an attempt at salvage cystectomy for tumours in which the treatment fails.

Several large RCTs have clearly shown that many patients with invasive bladder cancer benefit from neoadjuvant systemic chemotherapy prior to radical cystectomy. The most popular regimen currently in use worldwide is gemcitabine and cisplatin, as this protocol offers similar efficacy to alternatives such as methotrexate/vinblastine/doxorubicin/cisplatin (MVAC), but with fewer associated toxic effects. In 2012, the addition of paclitaxel to gemcitabine and cisplatin was evaluated in the neoadjuvant and metastatic settings by Bellmut *et al.*[8] The addition of this agent improved response duration by 3.1 months, although this effect was not statistically significant. However, the gemcitabine/cisplatin/paclitaxel regimen is complicated to administer and was associated with a higher rate of neutropaenic sepsis, suggesting that the benefit in response might not be enough to overcome these hurdles in everyday practice.

Currently, no validated therapies are available for use in patients with advanced bladder cancer that do not respond to first-line chemotherapy. This situation might begin to change as targeted agents are tested in this context. In 2012, Necchi *et al.*[9] reported a phase II evaluation of pazopanib—an antiangiogenic agent targeting the vascular endothelial growth factor (VEGF) axis—in patients with relapsing or chemorefractory bladder cancer. Pazopanib was used as a third-line (or higher) treatment in half of the patients included in the study and was associated with a partial response in 17% of participants. As patients had already relapsed or were refractory to existing chemotherapy, they were continued on pazopanib until disease progression or withdrawal due to toxicity (three patients developed fistulae attributed to a drug response). Given the paucity of treatment options for patients with refractory metastatic disease, the evaluation of targeted therapies such as pazopanib must be welcomed.

The case for individualized targeted therapies in bladder cancer was further advanced by a high-quality case report published by Iyer *et al.*[10] This group profiled the cancer genome of a patient with metastatic disease who achieved a dramatic durable response to everolimus. The tumour was found to have inactivating mutations of the *TSC1* (tuberous sclerosis complex 1) and *NF2* (neurofibromatosis type 2) genes. Loss of these genes is known to induce cellular dependency on the mTORC1 (mammalian target of rapamycin) complex, which is the target of everolimus. Mutations in *TSC1* were present in another cohort of patients with partial responses to everolimus and absent in those with no response. Based on the results, it might be possible in the future to select patients for everolimus treatment if tumours can be screened for these mutations. This report represents one of the first to integrate molecular tumour analysis with pharmacological selection in bladder cancer. This approach is commonplace in many other cancers, such as lung, colorectal and breast, and represents the probable long-term future of chemotherapy.

In summary, several reports published in 2012 challenged the current standards of care for non-muscle-invasive, muscle-invasive and advanced bladder tumours. We wait to see how these reports are replicated by others and to what extent they alter the outcomes for affected patients. However, the urological community urgently needs biomarkers that accurately stratify outcomes in high-risk non-muscle-invasive cancers and that can monitor the success of radical approaches for advanced disease, as well as increasingly effective systemic agents to reduce bladder-cancer-related mortality.

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Competing interests
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PROSTATE CANCER IN 2012

Paradigm shifts in prostate cancer diagnosis and treatment

Nathan A. Hoag and S. Larry Goldenberg

2012 was a year of global controversy surrounding PSA screening—updated results were reported from the two largest trials and the US Preventative Services Task Force (USPSTF) released a Grade D recommendation against screening. Other data supported active surveillance for low-risk prostate cancer, whereas radical prostatectomy was shown to benefit those with intermediate-risk and high-risk disease.

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Key advances

- Updated data from the European Randomized Study of Screening for Prostate Cancer (ERSPC) trial[1] showed a durable reduction in prostate cancer mortality in men who underwent PSA screening
- Updated data from the Prostate, Lung, Colorectal, and Ovarian (PLCO) screening trial[2] showed a continued lack of survival benefit associated with prostate cancer screening
- In the Prostate Cancer Intervention versus Observation Trial (PIVOT), radical prostatectomy offered no survival benefit over observation in men with low-risk prostate cancer, with benefits in overall and cancer-specific survival for intermediate-risk and high-risk disease[7]
- Intermittent androgen deprivation therapy shows noninferiority compared with continuous treatment, and is associated with an improvement in quality of life[9]

In 2012, several papers and editorials were published addressing PSA screening. Two large trials were intended to definitively answer the screening question, but, in fact, only contributed to the confusion owing to discordance in their findings, recommendations, and viewpoints. Firstly, the European Randomized Study of Screening for Prostate Cancer (ERSPC) reported 11-year follow-up results.[1] A total of 72,891 men were randomized to undergo screening and 89,152 to the control group. Control patients were not offered routine screening and, as such, the ERSPC was the first true randomized trial in which there was at least a 50% difference in the rates of screening when comparing the screened to the nonscreened cohorts. After 11 years, a total of 6,963 prostate cancer cases were diagnosed in the screening arm (9.6%) and 5,396 in the control arm (6.0%), with a 21% reduction in relative risk of death from prostate cancer; 29% after correction for noncompliance ($P=0.001$). At this 11-year follow-up point, 673 men in the compliant group would need to be offered screening and 33 cases of prostate cancer detected to save one death attributed to prostate cancer.

Secondly, researchers from the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial presented their 13-year follow-up data and again demonstrated no statistically significant cancer-specific survival benefit of organized screening compared with opportunistic screening as part of routine patient care.[2] Cumulative prostate-cancer-specific mortality rates were 3.7 and 3.4 deaths per 10,000 person-years for the screening and control arms, respectively (RR 1.09, 95% CI 0.87–1.36, $P=$NS).

Limitations and criticisms exist for both studies, with the oft-mentioned issues that, compared to ERSPC, the PLCO trial had a higher rate of contamination in the control arm (PSA tests carried out outside of the study protocol), was of smaller size, had a higher PSA cut-off point for biopsy, and a higher rate of prerandomization screening.[3] Neither study had overall survival as an end point, so prostate-cancer-specific mortality reductions might only be important in men with long life expectancies at time of screening. Furthermore, the long-term impact of PSA screening might only be revealed by future analysis of the number of quality-adjusted life years gained or lost, which will be linked to the role of active surveillance as a means of decreasing the harms of overtreatment. Future analyses need to examine issues such as selection of appropriate men for screening based on age and general health, screening intervals, optimizing the use of PSA, combining PSA with other biomarkers and imaging modalities, the effect of screening on the incidence of metastases and clarification of the data regarding screening younger men (≤54 years of age).

Based on their conclusion that the harms of screening outweigh the benefits, the US Preventative Services Task Force (USPSTF) recommended against PSA screening, retaining a grade D recommendation ("there is moderate or high certainty that the service has no net benefit or that the harms outweigh the benefits").[4] The task force reviewed data from the available prostate cancer screening trials; however, the USPSTF interpretation of the two largest screening trials—ERSPC and PLCO—has been a contentious issue, and one that garnered substantial attention in the media this year. Criticisms have been raised over their interpretation of the ERSPC trial in deciding how clinically relevant the modest survival benefit attributed to screening actually is, and in deciding that the harms of screening outweigh benefits.[5]

As mentioned previously, neither of the large trials were designed with overall survival as an end point and the USPSTF meta-analysis itself included poor quality evidence, failed to weigh studies according to their significance and did not consider the 40% reduction in prostate cancer mortality that has occurred in the USA since the advent of PSA testing. The USPSTF report emphasized the challenges of overdiagnosis and overtreatment but did not acknowledge the dramatic 44% mortality benefit in the healthy patient subset within the PLCO, with a number needed to treat (NNT) of 5 as reported by Crawford *et al.*[6] Screening for early-stage disease remains relevant in 2012, but the substantial time, energy, and resources directed towards this debate are detracting from more pertinent issues. The focus of discussion should shift away from whether (or not) to screen and towards risk stratification, prognostication and management of the overtreatment problem.

This past year we have seen progress made in understanding active surveillance as a standard-of-care for low-risk prostate

cancer. For the Prostate Cancer Intervention versus Observation Trial (PIVOT), 731 US veterans were randomized between 1994 and 2002 to receive either radical prostatectomy or observation for localized prostate cancer.[7]

Men were stratified into low-risk, intermediate-risk, and high-risk groups according to D'Amico criteria. PIVOT showed that overall survival for all risk groups was 2.9% higher and cancer-specific survival was 2.6% higher in surgically treated patients versus observation at the 10-year median survival mark, though this difference did not attain statistical significance.[7]

For those with low-risk disease, radical prostatectomy was associated with a 15% higher all-cause mortality than active surveillance although, again, this result was not statistically significant.

The most prominent overall survival benefit of 12.6% (relative reduction of 31%) was observed for patients with intermediate-risk tumours who underwent surgery compared to observation (HR 0.69; 95% CI 0.49–0.98). Patients with high-risk tumours undergoing radical prostatectomy had a non-significant reduction in all-cause mortality of 6.1% compared with observation but a significantly lower prostate-cancer-specific mortality (9.1% versus 17.5%, $P = 0.04$).

No prostate-cancer-specific benefit was observed in those men with PSA <10 ng/ml ($P = 0.82$), low-risk tumours ($P = 0.54$), or intermediate-risk tumours treated with surgery compared with observation ($P = 0.12$). Radical prostatectomy was not without perioperative morbidity, with 21.4% of men in PIVOT suffering complications and significantly higher incidences of erectile dysfunction and urinary incontinence in those men who underwent surgery.[7]

This latest report from PIVOT is one of the key papers of 2012, in that it showed the benefit of radical prostatectomy for patients with intermediate-risk and high-risk disease. Owing to the fact that the study was an intent-to-treat analysis with a high rate of noncompliance and because the recruited participants had a high (35% at 12 years) postdiagnosis other-cause mortality rate, it probably underestimates the true long-term treatment benefit of surgery, especially for intermediate-risk and high-risk disease. However, it also provides evidence that low-risk prostate cancer can be observed in the appropriate patient and supports the concept that active surveillance could solve the screening controversy by deflecting the current focus on overdetection and minimizing the overtreatment of potentially indolent disease.

Intermittent androgen deprivation therapy (ADT) has been well studied since 1995, when the first phase II study established its clinical application.[8] In 2012, a landmark study was published by Crook *et al.*[9] ascertaining noninferiority of intermittent ADT (8-month treatment cycles) versus continuous ADT for patients in whom localized prostate cancer has not been successfully treated with radiation therapy.[9] Their findings demonstrated a median survival of 8.8 years in the intermittent treatment group compared with 9.1 years in the continuous treatment group, with a hazard ratio of 1.02 (95% CI 0.86–1.21, $P = 0.009$). Furthermore, no statistically significant difference was observed in disease-specific survival, with 18% and 15% disease-related deaths in the intermittent and continuous treatment groups, respectively.[9] Although the quality-of-life differences were not as prodigious as might have been expected, there were statistically significant improvements in scores for hot flashes ($P < 0.001$), desire for sexual activity ($P < 0.001$), and urinary symptoms ($P = 0.006$).[9] This study's robust design and long follow-up duration should tip the balance in favour of intermittent therapy as standard-of-care for men with advanced prostate tumours.

2012 saw several key advances in the field of prostate cancer screening and treatment. Looking towards the future, the importance of screening in detecting early-stage cancer will be confirmed when our diagnostic and prognostic tools gain substantially better sensitivity and negative predictive values. Multiparametric MRI and targeted biopsy molecular characterization[10] and new biomarkers have the potential to transform management of low-risk disease by enabling wider acceptance of therapeutic approaches such as active surveillance and focal therapy. We look forward to further advances in 2013.

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Competing interests
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KIDNEY CANCER IN 2012

New frontiers in kidney cancer research

Kriti Mittal and Brian Rini

2012 has been an exciting year for kidney cancer research. From the FDA approval of a new targeted agent to revival of interest in immunotherapy, several interesting studies were published that generated considerable interest.

Mittal, K. & Rini, B. *Nat. Rev. Urol.* **10**, 70–72 (2013); published online 15 January 2013;
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The beginning of 2012 heralded the FDA approval of axitinib, the latest addition to the family of tyrosine kinase inhibitors approved for treatment of metastatic renal cell carcinoma (mRCC). Approval was based on the results of the pivotal phase III

AXIS trial. A potent vascular endothelial growth factor receptor (VEGFR) inhibitor, axitinib inhibits VEGFR-1, VEGFR-2 and VEGFR-3 at subnanomolar concentrations.

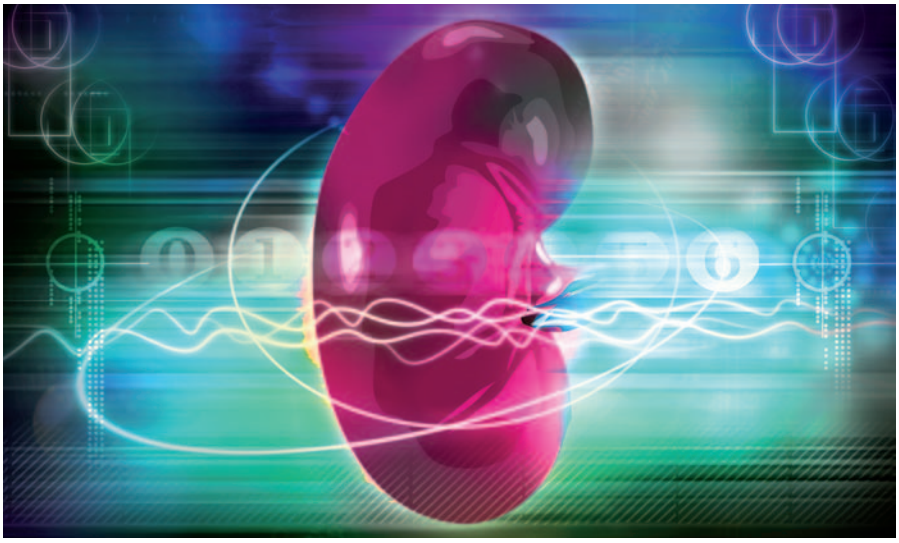
For the AXIS trial,[1] published at the tail end of 2011, 723 patients who were refractory to one previous systemic treatment were randomized to receive either 5 mg axitinib or 400 mg sorafenib twice daily. The primary end point was independently assessed progression-free survival (PFS), which was found to be 6.7 months for patients who received axitinib versus 4.7 months for those treated with sorafenib (the hazard ratio for disease progression or death was 0.665; $P<0.0001$). Objective response rate (ORR) was 19.4% for axitinib versus 9.4% for sorafenib ($P=0.0001$).

Like other VEGFR inhibitors, the main all-grade toxicities observed in over 30% of patients treated with axitinib were diarrhoea, hypertension, fatigue, nausea, anorexia and dysphonia. Myelosuppresion, hand foot syndrome and cutaneous toxicities were less common with axitinib than with traditional tyrosine kinase inhibitors. Common laboratory abnormalities included anaemia, hypothyroidism, lymphopenia and hypocalcaemia.

This was the first study to compare two active targeted agents for mRCC and these data highlight the robust efficacy of axitinib as second-line agent for mRCC. Additional clinical trials are underway to investigate axitinib treatment in patients with previously untreated mRCC to evaluate its role as a first-line therapy.

Another study reported in 2012 contributed to our knowledge of the optimal treatment schedule of sunitinib. In the first trial to investigate the effect of dose schedule on sunitinib treatment, Motzer *et al.*[2] compared the standard schedule (50 mg sunitinib daily for 4 weeks followed by a 2-week treatment break) with continuous daily dosing of 37.5 mg in treatment-naive patients with clear-cell mRCC.[2] This multicentre phase II study evaluated the impact of dose schedule on time to progression (TTP), which was the primary outcome, and ORR, overall survival, safety, tolerability and patient-reported outcomes as secondary end points.

The results of this study favoured the standard dose schedule over continuous daily dosing, with increased median PFS (8.5 months versus 7 months) and TTP (9.9 months versus 7.1 months), although these differences did not reach statistical significance ($P=0.07$ and $P=0.09$, respectively). Continuous dosing did not translate into superior relative dose intensity (91% for standard dosing; 78% for continuous dosing) or improved tolerance. ORR was 32% in the standard dose arm compared with 28% for those who received continuous dosing ($P=0.444$) and the incidence of all-grade toxicities was comparable between groups.



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As might be expected, patient-reported outcomes—according to standard scoring systems—were better after the 2-week break than at day 28 for the standard dosing arm. The standard dosing arm was also significantly better than the continuous dosing arm in terms of time to deterioration (HR 0.77; $P=0.034$). Consistent with existing evidence,[3] this study highlights the importance of dose intensity for the maintenance of antitumour efficacy, and reinforces the conventional sunitinib schedule as the standard of care.

Also in 2012, Gerlinger and colleagues[4] offered new insight into the genomic heterogeneity of mRCC. Exome sequencing, chromosomal aberration analysis and ploidy profiling of primary and metastatic lesions were performed in four patients with mRCC undergoing cytoreductive nephrectomy. While the von Hippel Lindau (*VHL*) gene was mutated in all regions of the tumours analysed, other mutations had varying regional distributions that were spatially separated. Considerable differences were revealed, not only between primary and metastatic sites, but also within primary tumour specimens from a single patient. For example, in one patient, only 55% of all detected somatic mutations were present in the biopsy specimen, and only 34% of all mutations in the nephrectomy specimen were detected in all regions.

The findings of this study have considerable clinical relevance, signifying that a single histological sample might not adequately portray the entire spectrum of genetic aberrations and allelic imbalances in a heterogeneous tumour. Personalized therapy based on gene signature profile is already gaining momentum for other solid malignancies, such as lung cancer. Although such personalization is not directly relevant to the currently available therapies for mRCC (as none are directed toward specific genetic mutations) these data compel us to reassess our vision for future therapeutic approaches for mRCC.

Emerging data from two phase I studies reported in 2012 have revived interest in immune therapy for mRCC.[5,6] Programmed cell death protein 1 (PD-1) is an immune check-point inhibitor expressed by activated T cells. PD-1 interacts with its ligands PDL-1

Key advances

- Results of the phase III AXIS trial demonstrate the superiority of axitinib over sorafenib, in terms of progression-free survival (PFS), as second-line therapy for metastatic renal cell carcinoma (RCC)[1]
- The conventional dosing schedule of sunitinib (50 mg daily for 4 weeks followed by a 2-week treatment break) provides superior PFS and time to progression to continuous daily dosing in the first-line setting[2]
- Intratumour heterogeneity has been demonstrated in primary and metastatic lesions of patients with RCC[4]
- Antibodies against programmed cell death protein 1 and its ligand have demonstrated efficacy in the treatment of previously treated advanced RCC[5,6]

and PDL-2, which are expressed on tumour cells, leading to attenuation of the cytotoxic T-cell response. BMS-936558 is an anti-PD-1 monoclonal antibody that inhibits the interaction between PD-1 and its ligands.

This year, Topalian and colleagues[5] published findings of a phase I study of BMS-936558 in 296 patients with various solid tumours, including 34 patients with mRCC. Patients had received 1–5 previous systemic treatments; 59% of the mRCC cohort had received prior immunotherapy and 74% had been treated with antiangiogenic agents. BMS-936558 was administered intravenously once every 2 weeks of each 8-week treatment cycle, for up to 2 years. Three dose levels were tested: 1 mg/kg, 3 mg/kg and 10 mg/kg. The drug was tolerated well, with drug-related grade 3 or 4 adverse effects observed in only 14% of patients. Objective response was demonstrated for patients with non-small-cell lung cancer, melanoma or RCC, with ORRs of 24% and 31% in patients with mRCC assigned to receive 1 mg/kg and 10 mg/kg, respectively. The PFS rate at 24 weeks was 56% for patients with mRCC. In a retrospective subset analysis, objective response was identified in 36% of the patients who had PDL-1-expressing tumours, and in none of the patients who lacked expression of this ligand (specimens were available from 42 patients with any tumour type).

Common treatment-related adverse effects included fatigue, rash, diarrhoea, pruritus, decreased appetite and nausea. Immunologic drug-related adverse effects of particular interest included pneumonitis, vitiligo, colitis, hepatitis, hypophysitis and thyroiditis. The incidence of grade 3 or 4 adverse effects was 6% in the overall cohort, and three pneumonitis-related deaths were reported, all three of which occurred in patients with cancers other than RCC. Overall, the incidence of severe adverse effects was low, and therefore this drug is expected to be tested further in larger clinical trials.

In a companion study, Brahmer and colleagues[6] investigated an anti-PDL-1 antibody, BMS-936559, which inhibits binding of PDL-1 to PD-1 or CD80. 207 patients were enrolled in this phase I study, including 17 individuals with previously treated mRCC. Two of these patients with mRCC reported an objective response, leading to an ORR of 12%. Objective responses were durable for both agents, many lasting for a year or more. Together, these data illustrate that immune check-point inhibitors could be an effective therapeutic strategy in RCC and additional trials are in progress.

To conclude, RCC research in 2012 has continued to evolve at a rapid pace. Hopefully, the developments outlined above will in turn set the stage for more novel therapeutic advances in the future.

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Competing interests
B. Rini declares an association with the following company: Pfizer. See the article online for full details of the relationship. K. Mittal declares no competing interests.

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BPH IN 2012

Novel agents in treatment of BPH

Bilal Chughtai and Alexis Te

2012 in BPH has been characterized by studies investigating treatment options for BPH-associated lower urinary tract symptoms (LUTS). Onabotulinumtoxin A and saw palmetto have both been shown to be no more effective than placebo. However, in a positive move, tadalafil received FDA approval for LUTS treatment.

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During 2012, we have seen a number of significant updates in agents commonly used to treat BPH. These agents include those previously used as phytotherapy for BPH, or for the treatment of other urological conditions including neurogenic bladder and erectile dysfunction.

The Complementary and Alternative Medicine for Urological Symptoms (CAMUS) trial is of particular clinical importance as most US physicians have been reluctant to discuss or recommend phytotherapy for patients with BPH, owing to the sparsity of published reports in peer-reviewed medical literature.[1] One of the most commonly used phytotherapies is *Serenoa repens* (saw palmetto); extract of the saw palmetto berry was the third most common herbal remedy sold in the USA in 2006.[2,3]

The CAMUS trial included 357 men at 11 sites, who were randomized to receive either 320 mg, 640 mg, or 960 mg of ethanolic saw palmetto in an escalating fashion or placebo.[1] The primary outcome of the study was change in American Urological Association Symptom Score (AUA-SS); secondary outcomes included nocturia, flow rate, postvoid residual volume, sexual function, and pain scores. The study found no difference in the escalating dose of saw palmetto compared to placebo. In addition, there were no differences between secondary outcomes. This trial thus enables physicians to now offer their patients evidence-based recommendations regarding herbal medications.

As part of the CAMUS trial, a subset analysis was also performed to investigate

an association between sleep disturbances and lower urinary tract symptoms (LUTS).[4] Sleep disturbances were measured using the Jenkins sleep scale and a combined analysis was performed on the whole cohort. Notably, there was a significant association between AUA-SS and sleep disturbances irrespective of nocturia. Furthermore, improvements in LUTS without changes in nocturia led to improvements in sleep quality. Overall, LUTS were more predictive of sleep disturbances than nocturia alone. This result suggests that sleep quality and LUTS might have a common aetiology that could be targeted to improve both conditions. Thus, it becomes imperative for urologists to search for other causes of sleep disturbances when dealing with nocturia, such as obstructive sleep apnoea.

In addition to trials of herbal medications, there has been a push to test agents that have been used in other areas of urology for LUTS. Onabotulinumtoxin A—which was first used for cosmetic purposes then for treatment of neurogenic bladder—has been applied to BPH. Several pilot studies and dose finding studies have been carried in the realm of BPH and a number of single-arm trials have shown subjective improvements in LUTS with onabotulinumtoxin A on the prostate when administered by either a transrectal or transperineal route. In 2012, we saw the report of a multicentre, double-blind, phase II dose-finding study, in which 380 men were randomized to receive placebo or onabotulinumtoxin A, via a transperineal or transrectal route.[5] The primary outcome was change in AUA-SS. All patients experienced an improvement all parameters, including AUA-SS and Q_{max} (maximum flow rate) using 100, 200 or 300 U onabotulinumtoxin A or placebo. A substantial placebo effect was noted after both transperineal and transrectal injections. Despite these results, there was also a significant reduction in AUA-SS in patients not exposed to α-blockers. These patients might, therefore, represent a target population who could benefit from this agent.

2012 has also seen the phosphodiesterase type 5 (PDE5) inhibitor tadalafil gain FDA approval for treatment of LUTS secondary to BPH. Several trials have shown subjective benefit of tadalafil for LUTS,[6–9] and this year Gacci *et al.*[10] reported a meta-analysis that pooled data from 3,214 men. Seven of the trials included in the analysis compared PDE5 inhibitors with placebo treatment and five compared a combination treatment using PDE5 inhibitors and α-blockers with placebo.[10] Over a median follow-up period of 12 weeks, the data showed a significant improvement in erectile function based on the International Index of Erectile Function (IIEF) and AUA-SS. Although there were statistically significant improvements in subjective parameters, there were no improvements in objective parameters such as Q_{max}. Further trials will need to be designed to get a better understanding of objective changes in LUTS with the use of PDE5 inhibitors.

These trials have answered some important questions for clinicians in 2012. Specifically, the CAMUS trial has provided level 1 evidence on the use of alternative therapies, which is especially important considering that saw palmetto is so commonly used by men with LUTS secondary to BPH, and that there have previously been very little data regarding its safety and efficacy. Physicians should counsel their patients about the results of these studies, specifically explaining that saw palmetto has a good safety profile, but that it provides no demonstrable benefit in the realm of LUTS. In addition, onabotulinumtoxin A has now demonstrated efficacy in several different clinical contexts—where it previously had a role mainly in cosmetic procedures, it now finds uses in gastroenterology as well as several genitourinary indications. However, once again, level 1 evidence suggests that prostatic application of this agent does not lead to a significant improvement of LUTS, regardless of the route of delivery. In 2011, the FDA approved the use of tadalafil for the treatment of LUTS, despite the fact that PDE5 inhibitors have demonstrated improvements in subjective symptoms but no improvement in objective parameters such as Q_{max} or urodynamic parameters. This dichotomy of subjective versus objective findings in patients with LUTS will require further investigation. Overall, 2012 has seen several important trials and manuscripts in the realm of BPH and voiding dysfunction. BPH therapies are evolving and, with the application of novel and targeted agents, we hope to see better therapies and interventions in the coming years.

Key advances

- The CAMUS Trial shows that *Serenoa repens* (saw palmetto) extract is no more effective than placebo for treating BPH-associated lower urinary tract symptoms (LUTS)[1]
- Subset analysis of the CAMUS trial data suggests a common aetiology between LUTS and sleep quality, which might indicate a potential common therapeutic target for both disorders[4]
- Onabotulinumtoxin A is shown to improve LUTS parameters in a multicentre, double-blind, phase II dose-finding study, but a substantial placebo effect is also noted and the study concludes that there is no benefit over placebo[5]
- Tadalafil, a phosphodiesterase inhibitor usually used for treating erectile dysfunction, is shown to improve LUTS[10] and receives FDA approval for this indication

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Competing interests
The authors declare no competing interests.

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INFECTION IN 2012

Mixed results of pre-exposure prophylaxis for HIV prevention

Ronald H. Gray and Maria J. Wawer

In 2012, three trials of antiretroviral pre-exposure prophylaxis (PrEP) were reported, with conflicting results. Taken together with data from 2010 we now have two trials that showed no benefit and four studies that demonstrated moderate-to-high efficacy. Accordingly, it seems that PrEP might have a limited role for HIV prevention in individuals at high-risk of infection.

Gray, R. H. & Wawer, M. J. *Nat. Rev. Urol.* **10**, 74–75 (2013); published online 15 January 2013; doi:10.1038/nrurol.2012.259

The use of antiretroviral drugs for pre-exposure prophylaxis (PrEP) to prevent HIV acquisition in exposed but uninfected individuals has gained considerable interest over the last few years. The concept of PrEP is especially appealing as a means of protecting vulnerable women (who might have limited access to other methods of protection, such as condoms) and men who have sex with men.[1] Six trials of PrEP have now been published altogether (Table 1), three of which were reported in 2012 with conflicting results.

In 2010, the randomized Centre for the AIDS Programme of Research in South Africa (CAPRISA) 004 trial was the first to report the efficacy and safety of tenofovir disoproxil fumarate (TDF) 1% vaginal gel in 445 women at risk of contracting HIV.[2] Participants were instructed to apply the gel within a 12 h period before intercourse and as soon as possible within 12 h after sex. HIV incidence after 30 months of follow-up was 5.6 cases per 100 person-years in the intervention arm and 9.1 cases per 100 person-years in the placebo arm, with an overall reduction of HIV acquisition of 39%. Among women who reported use of the TDF gel for more than 80% of sex acts, HIV reduction was 54%, and lower adherence was associated with lower efficacy. No serious adverse effects were reported for the TDF gel and no drug-resistant virus was detected among gel users who did acquire HIV. Furthermore, TDF gel was also found to reduce herpes simplex virus type 2 infection by 51%.

These promising results led to a randomized trial for men who have sex with men (the iPrex study), using oral tenofovir and emtricitabine (TDF-FTC, marketed as Truvada® by Gilead Sciences [USA]). Also reported in 2010, the iPrex study involved randomization of patients to receive daily TDF-FTC ($n = 1,251$) or placebo ($n = 1,248$) and showed a 44% reduction in HIV acquisition in the intervention group compared with those who received placebo.[3] These results were considered to be promising for protection of men who have sex with men. However, poor adherence—measured by detection of the study drug in blood—was associated with HIV acquisition in the TDF-FTC arm, and drug-resistant mutations were detected in two patients who were infected at the time of study enrolment. A 1% reduction in bone mineral density was reported in TDF-FTC recipients compared with those in the placebo arm.

In late 2011 and in 2012, however, we learnt the disappointing results of two more TDF-FTC trials. The FEM-PrEP trial[4] was conducted in a cohort of 1,950 African women at high-risk of acquiring HIV who took either daily oral TDF-FTC or placebo. The study was closed prematurely owing to lack of efficacy, potentially as a result of poor drug adherence thought to be related in part to adverse effects, such as nausea and vomiting, possibly as a consequence of hepatic and renal abnormalities.[4] Similarly, the microbicide trials network 003 trial (also known as VOICE; Vaginal and Oral Interventions to Control the Epidemic), which included 5,021 African women randomized to receive daily oral TDF, daily oral TDF-FTC, daily vaginal TDF gel or placebo was also stopped early.[5] The authors reported higher rates of drug discontinuation owing to hepatic or renal abnormalities in the TDF-FTC group than the other study arms, and adherence to all study regimens was poor, as indicated by the low proportion of participants (40%) with detectable serum levels of the drugs.[5]

On a more positive note, results of the TDF2 trial of heterosexual men and women exposed to HIV in Botswana who were randomized to receive daily oral TDF-FTC ($n = 611$) or daily placebo ($n = 608$) were published in 2012.[6] Although enrolment to the study was closed prematurely because of low retention rates, the investigators continued to follow-up previously enrolled participants. In a modified intention-to-treat analysis, the rates of HIV acquisition were 1.2 cases per 100 person-years in the TDF-FTC

Table 1 | Pre-exposure prophylaxis to prevent HIV transmission

Study	Population	*n*	Drug regimen	Efficacy for HIV prevention
CAPRISA 004 (South Africa)[2]	Women at high risk	889	Vaginal TDF gel used before and after coitus	39%
iPrex (Brazil, Equador, Peru, South Africa, Thailand, USA)[3]	Men who have sex with men and transgender women	2,499	Daily oral TDF-FTC	44%
FEM-PrEP (Kenya, South Africa, Tanzania)[4]	Heterosexual women at high risk	1,950	Daily oral TDF-FTC	Trial stopped for lack of efficacy
VOICE/MTN 003 (South Africa, Uganda, Zimbabwe)[5]	Women	5,021	Daily oral TDF, TDF-FTC or vaginal TDF gel	Oral and vaginal gel TDF stopped for lack of efficacy; TDF-FTC continuing
TDF2 Study (Botswana)[6]	Heterosexual men and women	1,299	Daily oral TDF-FTC	62%
Partners PrEP Study (Kenya, Uganda)[7]	HIV-discordant couples	4,758	Daily oral TDF or TDF-FTC	TDF 63% TDF-FTC 75%

Abbreviations: CAPRISA, Centre for the AIDS Programme of Research in South Africa; iPrex, Pre-exposure Prophylaxis Initiative; MTN, microbicide trials network; PrEP, pre-exposure prophylaxis; TDF, tenofovir disoproxil fumarate; TDF-FTC, tenofovir disoproxil fumarate and emtricitabine; VOICE, Vaginal and Oral Interventions to Control the Epidemic.

arm, compared with 3.1 cases per 100 person-years in the placebo arm, with an efficacy for TDF-FTC of 62.2%. The TDF-FTC arm was not without adverse effects though, with participants reporting higher rates of nausea, vomiting and dizziness than those in the placebo arm. TDF-FTC was also associated with significant declines in bone mineral density.

Finally, the Partners PrEP trial of 4,758 HIV-discordant heterosexual couples from Kenya and Uganda also yielded positive data this year.[7] For this study, the HIV-infected partners did not receive antiretroviral therapy (ART) and HIV-uninfected partners were randomized to receive daily oral TDF, TDF-FTC or placebo. In 62% of couples, the uninfected partner was male. HIV incidence was 0.65 cases per 100 person-years in the TDF arm, 0.50 cases per 100 person-years in the TDF-FTC arm and 1.99 cases per 100 person-years in the placebo group. The efficacy for HIV prevention was 67% with oral TDF alone and 75% with TDF-FTC, although the difference between these two drug regimens was not statistically significant ($P = 0.23$). Antiretroviral drug resistance was detected in two of eight participants who were found to be infected at the time of enrolment. There were no statistically significant differences in rates of adverse events between study arms. These findings are promising for HIV-discordant couples in which the uninfected partner is at high risk of HIV infection.

To summarize, new research on the efficacy of PrEP published or reported up to 2012 is mixed, with two trials showing no benefit, and four showing moderate-to-high efficacy. Previous evidence suggests that high adherence is needed to maintain protection and it is likely that poor compliance accounts for the two trials demonstrating no benefit. Further research on different modes of administration (topical versus oral) in diverse settings is clearly needed.[8] One further trial (Follow-on African Consortium for Tenofovir Studies; FACTS 001) was initiated in South Africa in 2011 to assess TDF vaginal gel applied around the time of coitus, but no results are available from this study yet.

Adverse effects, including viral resistance, depletion of bone mineral density, drug-related nausea and vomiting and metabolic abnormalities were noted in a number of the trials and there are also concerns about interactions between TDF-FTC and hormonal contraceptives. These considerations will probably limit the utility of PrEP for HIV prevention programmes and restrict its use to limited subgroups at high risk of HIV exposure.[1] Such subgroups might include uninfected partners in serodiscordant relationships with an infected partner who either is ineligible for, or refuses to take, ART, and for HIV-negative men who practice unprotected anal intercourse.[1] However, in the USA the estimated cost of Truvada® is approximately US$13,900 per patient per year. Costs might be lower in developing countries if cheaper generic drugs become available, but the need for prolonged use could be a drawback for overburdened programmes with limited resources.[9]

PrEP is a potentially useful preventive intervention for select subgroups of HIV-uninfected persons at high risk of HIV infection. However, it is not a panacea and is certainly not a substitute for safe sexual practices, such as consistent condom use or partner reduction, nor should it displace proven prevention strategies, such as male circumcision or ART both for therapeutic benefit and prevention of transmission.

Key advances

- Evidence for the efficacy of antiretroviral pre-exposure prophylaxis published in 2012 has been mixed
- Two trials of tenofovir disoproxil fumarate (TDF) in African women have been stopped early owing to poor efficacy, low adherence and adverse effects[4,5]
- The TDF2 trial of heterosexual men and women exposed to HIV in Botswana revealed an efficacy for TDF and emtricitabine (TDF-FTC) of 62.2%[6]
- The Partners PrEP trial of HIV-discordant heterosexual couples from Kenya and Uganda yielded efficacies for HIV prevention of 67% for oral TDF alone and 75% for TDF-FTC[7]

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Competing interests
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STONES IN 2012

Epidemiology, prevention and redefining therapeutic standards

Andreas Neisius and Glenn M. Preminger

Interesting data on kidney stone prevalence and metabolic risk factors for stone formation in children were published in 2012, suggesting that we must invest more time and resources in epidemiological research. Further advances in endoscopic technology will hopefully lead to lower morbidity and better outcomes for stone removal procedures.

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2012 was a year of progress in the understanding and management of urolithiasis. Improved technologies have facilitated the performance of ureteroscopy and percutaneous nephrolithotomy (PNL) and an increasing number of studies published

Key advances

- Higher prevalence of kidney stones is probably related to dietary and lifestyle factors, although only high levels of sodium intake are a proven risk factor for kidney stone formation[1]
- Hypercalciuria is the principal urine risk factor for calcium stone formation in children[5]
- Although percutaneous nephrolithotomy (PNL) remains the gold standard for the management of complex renal calculi, flexible ureteroscopy with laser lithotripsy can be considered for stones >2 cm with high success and low complication rates in selected patients[8]
- PNL is a safe treatment option for stone removal with high success rates in experienced hands. Appropriate patient preparation and advances in endoscopic technology can significantly reduce complications[9]

over the last few years have enabled researchers to put together high-quality collaborative reviews and meta-analyses on this topic. Moreover, epidemiological and metabolic studies have helped to identify risk factors and medical treatments for recurrent nephrolithiasis.

After a break of 13 years, the National Health and Nutrition Examination Survey (NHANES) resumed the collection of cross-sectional data regarding kidney stone prevalance from the US population, and the results were published in 2012.[1] Between 2007 and 2010, 12,110 individuals were assessed to identify risk factors associated with the history of kidney stones. The findings revealed an overall prevalence of kidney stones of 8.8% (95% CI 8.1–9.5), indicating that 1 in 11 people in the USA are currently affected by stone disease, compared with only 5.2% (1 in 20 people) in 1994. Analysis also demonstrated that the prevalence of urolithiasis was higher for men (10.6% [95% CI 10.0–12.3]) than for women (7.1% [95% CI 6.4–7.8] $P<0.001$), and that white people had the highest likelihood of stone disease, compared with black individuals (OR 0.37, 95% CI 0.28–0.49; $P<0.001$) and with those of Hispanic origin (OR 0.60, 95% CI 0.49–0.73; $P<0.001$). Moreover, the prevalence of kidney stones was significantly higher for people with BMI >30 kg/m^2 (11.2%, 95% CI 10.0–12.3) than for those with BMI 18.0–24.9 kg/m^2 (6.1%, 95% CI 4.8–7.4; $P<0.001$).

Using multivariable models, investigators found that age, gender, race, socioeconomic status, obesity, diabetes and gout disease were strongly associated with a history of kidney stones. These findings suggest that the higher prevalence of kidney stones in the contemporary cohort is probably related to dietary and lifestyle factors, although analysis of dietary intake in the observed study population revealed that only high levels of sodium intake were a significant variable for stone formation. Given the previous evidence that dietary and lifestyle factors, such as the metabolic syndrome, can increase stone recurrence rates >50% within 5 years in a working age population,[2] it is hoped that these findings—along with other evidence from randomized controlled trials[3,4]—encourage researchers to focus on the role of lifestyle factors and secondary prevention of recurrent nephrolithiasis.

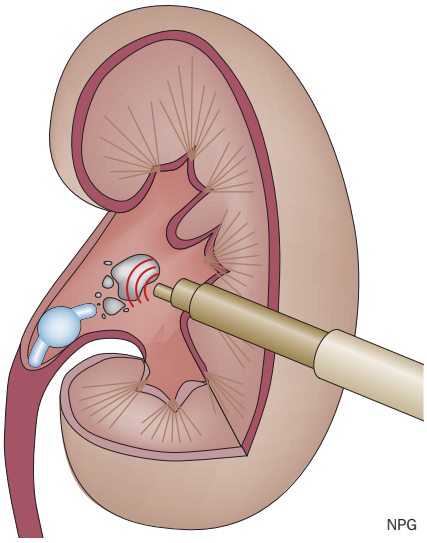
Another landmark study published in 2012 suggests that hypercalciuria is the only urine measure that is a significant risk factor for calcium stone formation in children and their siblings.[5] This situation is very different to the one in adults, for whom hyperoxaluria, hypocitraturia, abnormal urine pH and low volume have also been demonstrated to confer an increased risk of calcium stone formation.[6] For this study, 24 h urine specimens from 129 stone-forming children were compared with those from 105 non-stone-forming siblings and from 183 healthy children without a family history of stone disease (aged 6–17 years). Significant differences in daily calcium excretion were demonstrated between stone-forming children and both of the other patient groups, and also between non-stone-forming siblings and healthy children. Although stone-forming children tended to excrete higher levels of sodium than the other children, this difference did not reach statistical significance ($P>0.1$). Stone-forming children also demonstrated a reduced distance between the upper limit of metastability and supersaturation for calcium phosphate indicating an increased risk of stone crystallization. Given that hypercalciuria is known to be an inherited disorder,[7] it is not surprising that siblings of stone-forming children are more likely to excrete an elevated level of urine calcium than children with no family history of urolithiasis. Indeed, the fact that urinary calcium excretion is significantly higher in stone-forming children compared to their siblings underlines the hypothesis that hypercalciuria itself is a principal cause of urolithiasis.

Enhanced endoscopic procedures, such as digital flexible ureteroscopy combined with improved auxiliary measures (ureteral access sheaths and improved nickel–titanium devices) have been challenging traditional approaches (such as PNL) for the management of symptomatic renal calculi for some time. In 2012, high-level evidence was published that provides justification for urologists to manager larger renal stones with flexible ureteroscopy. A systematic review of flexible ureteroscopy and laser lithotripsy for renal stones >2 cm in diameter (mean stone size 2.5 cm) demonstrated a mean stone-free rate of 93.7%.[8] Nine studies including a total of 445 patients (460 renal units) were included in this meta-analysis. The high stone-free rate was achieved with an average of 1.6 procedures per patient and an overall complication rate of 10.1%. Subgroup analysis demonstrated a stone-free rate of 95.7% for stones that measured 2–3 cm in size compared with only 84.6% for stones >3 cm ($P=0.01$) and minor complication rates were similar at 14.3% and 15.4%, respectively. Major complications only occurred in the >3 cm group (0% versus 11.5%). However, these promising results must be evaluated against the limitations of this meta-analysis. Minor and major complications were not defined in any of the studies; therefore, no meaningful comparisons can be made. Similarly, stone-free rates were not adequately defined. In two studies, stone-free rate was defined as residual fragments <2 mm, in a further six studies it was defined as residual fragments ≤2 mm, and one study reported the 'true stone-free rate' (zero residual fragments), 0–2 mm fragments and the rate of occurrence of residual fragments measuring <4 mm. Moreover, all of the results were from observational cohort studies and from high-volume centres, which might have conferred a selection bias. Stone-free rates reported from high-volume centres are often superior to those expected in the general community. The authors conclude that flexible ureteroscopy with laser lithotripsy is a safe approach for the treatment of renal stones >2 cm, demonstrating a high success rate and a low complication rate in selected patients, but that PNL should still be considered the gold standard for the management of large renal calculi. For most patients, PNL should still be considered first-line treatment for large or complex renal calculi; however, these data suggest that for patients with contraindications to PNL (such as bleeding disorders, anticoagulation or morbid obesity), repeat session flexible ureteroscopy could

provide reasonable stone-free rates for the management of large renal stones.

Finally, results of a collaborative study of the incidence, prevention and management of complications of PNL were reported in 2012, and were found to support its use for complex renal stone management.[9] The epidemiology of complications associated with PNL is hard to evaluate owing to the lack of standardized definitions. For this study, Clavien grades[10] were assessed in 7,312 patients and specific complications were assessed in 11,929 patients from a total of 115 studies. The results demonstrate that Clavien level 0 complications were the most commonly reported, in 76.7% of patients. At the other end of the spectrum, Clavien level 4 complications (life threatening) were reported in 0.6% of patients and Clavien level 5 complications (mortality) were observed in 0.04% of the cohort.

Complications that are specific to percutaneous surgery were investigated more thoroughly, and it was found that septicaemia can be prevented by preoperative admission for intravenous antibiotics in patients with stones >2.5 cm, hydronephrosis or bacteriuria (level 1b evidence). Moreover, analysis revealed that pain or complications associated with the percutaneous nephrostomy tube can be avoided by performing tubeless PNL procedures or nephrostomy tract infiltration with local anaesthetics (level 1b evidence). The authors conclude that serious complications after PNL can be significantly minimized if experienced surgeons perform PNL for appropriate indications. Furthermore, antibiotic therapy directed by preoperative urine cultures, antibiotic prophylaxis in patients with sterile urine, and the establishment of reasonable percutaneous access are crucial to prevent



NPG

complications. These findings suggest that patients with high stone burden should be referred to high-volume centres to minimize the occurrence of complications. Further research is warranted to avoid systemic procedural complications.

In summary, these four papers published in 2012 help us to understand the increasing prevalence of urinary stones and provide guidance for the medical and surgical management of nephrolithiasis. Future research is needed into the lifestyle factors associated with stone formation, which could aid secondary prevention and lead to improved (noninvasive) management. With further advances in endoscopic technology, ureteroscopic and PNL techniques will no doubt continue to improve. In order to better assess the impact of advanced endoscopic techniques and to more accurately compare the results of different procedures, we must accept and utilize agreed-upon definitions of stone-free rates and surgical complications.

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Competing interests
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