Uses of the Polymerase Chain Reaction in Solid Tumor Oncology
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Good morning to all. I wish to thank Novartis for the invitation to this important meeting. I must disclose that I have several conflicts of interest, including: Novartis, Roche, BMS, AZ, that I can recall. I'm working on several more, I'll keep you posted.

During the next 12 minutes I will try to convey the importance of PCR in solid tumor oncology. My task is easier since many of the speakers yesterday aluded to key biomarkers obtained through PCR: Dr. Arrieta spoke extensively on EGFR, KRAS and EML4/ALK mutations for lung cancer; Dr. Simon told us about constitutive mutations of AKT and PI3K in breast cancer; Paulo Hoff and Dr. Juan O'Connor gave us a glimpse of the future in targeted therapy based on protein expression in neuroendocrine tumors, and GIST, respectively.

Tbe Polymerase Chain Reaction is a technique to amplify a single (or a few) copies of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. The invention of PCR is credited to Kary Mullis in 1983, for which he received the Nobel Prize in Chemistry in 1993. Key to the process was the discovery of termostabile DNA polymerase that is able to withstand high temperatures, with an optimal operating temperature of about 70 degress Celsius.

The process starts with double stranded DNA containing the sequence that we want to amplify. Oligonucleotide primers specific for target DNA fragments, nucleotide triphosphates and termstable DNA polymerase are ALL placed in a tube. Next, the temperature is raised to 95 degress Celsius so dsDNA denatures to ssDNA. The heat is decreased to about 60 degrees Celsius so the primers can anneal to the complementary ssDNA. The temperature is raised again to about 70 degrees Celsius so the Taq DNA polymerase starts DNA synthesis from the 3' end of the annealed primers. The Taq polymerase can synthesize about 1kb per minute. This first cycle doubles the target sequence. The cycle is repeated 20-30 times creating millions of copies. All this process can be automated, with temperature cycles carefully controlled. PCR is particularly effective for the amplification of medium-sized sequences (up to a few thousand bases).

RT-PCR uses the same technology to amplify mRNA, adding a Reverse Transcriptase that will synthesize DNA from RNA. RT-PCR is used for the evaluation of gene expression, and can be quite helpful in the evaluation of response in leukemia and in endocrinology.

PCR is invaluable for research. But I will devote a few minutes to its use in our daily practice in solid tumor oncology.

All oncologist are aware of the importance of the EGFR pathway in many solid tumors. The EGFR binds to its ligands, dimerizes in the cellular membrane. The TKs of the intracellular domain trigger a cascade, that includes activation of RAS, RAF, and other intracellular mediators.

The end result, causes proliferation, angiogenesis, blockage of apoptosis, etc. All these are important to the oncologic phenotype.

MOAs like cetuximab can target the EGFR, blocking its downstream actions. Tyrosine Kinase inhibitors like erlotinib or gefitinib can also block the cascade, as well.

Abnormalities in the EGFR pathway are frequent in oncology. EGFR overexpression is found in Pancreatic, Colorrectal and NSCL. Constitutively activated RAS mutations are found in the vast majority of pancreatic cancer, and about 30-40% of patients with NSCLC and colorrectal cancer. Constitutively activated RAF mutations are found in most melanoma patients, and in a minority of patients with colon and thyroid cancer. As can be easily surmised, constitutive activation of the downstream RAS or RAF render useless any strategy blocking the EGFR.

This is illustrated in this image, in which there is a KRAS mutation. The blockage of the EGFR with a MOA, like cetuximab, will not affect this RAS driven proliferation.

In the CRYSTAL study unseleceted, treatment naive, metastatic colorectal cancer patients were randomized between FOLFIRI chemotherapy or FOLFIRI + Cetuximab, an anti EGFR MOA.

The study was positive with a significant increase of PFS from 8 to 8.9 months, in favor of the cetuximab arm.

All the relevant outcomes were superior in the subgroup of patients with wild-type, that is unmutated, KRAS, that received cetuximab. The PFS was 9.9 months compared to 8.4 in the FOLFIRI only arm. The response rate was also higher in the cetuximab treated wtKRAS patients. Not shown is the fact that KRAS mutated patients did not derive any benefit from anti EGFR agents.

The use of cetuximb as a last line in mCRC is supported by this trial in which patients that had progressed to all available agents were randomized to cetuximab or BSC. The study was, again, positive with a median survival of about 6 months, 1.5 months more than the BSC arm.

If we select the wtKRAS patients we find a very nice 9 month OS in cetuximab treated patients in this trial. Not shown, cetuximab was of no benefit in patients with mutant KRAS.

In this graph we can observe the Lack of Effect of cetuximab in the BRAF mutated patients.

With just these two biomarkers, both of which are tested using PCR, we could potentially exclude about half of the mCRC patients NOT likely to derive any benefit from anti EGFR therapy.

These are not the only potential abnormalities in mCRC. Some of them amenable to evaluation using PCR technology.

Let's move to the NSCLC arena. Ten percent NSCLC patients have EGFR mutations in the TK domain. Mostly on Exon 19. From preclinical models it was postulated that these mutations increased the susceptibility to anti EGFR TKI.

Dr. Arrieta showed us yesterday the IPASS in NSCLC. A very courageous study in which mostly non-smoker asian patients were randomized to chemotherapy or gefitinib (another EGFR TKI).

As you can see, this graph is confusing. The green line has crossed over the orange line at some point. When that happens, statistitians suspect that there is more than ONE population. And they were right.

About 60% of the patients harbored an EGFR mutation.

As you can see, the PFS of mutated EGFR was superior in the gefitinib group. The converse was also true, patients with unmutated EGFR were more likely to derive benefit from conventional chemotherapy than to targeted therapy.

EGFR mutation analysis can be helpful, since the preconceptions that they occur only in female, non-smokers, and adenocarcinomas, can exclude a significant number of EGFR mutated patients that are male, smokers and with other histologies that could derive benefit from anti EGFR TKIs.

An impressive 70% response rate has been reported in a large cohort of EGFR mutated NSCLC patients treated with erlotinib (another EGFR TKI) in Spain.

The benefit of maintenance erlotinib in the SATURN trial, is observed mostly in the mutated EGFR subgroup of patients.

Time precludes me from discussing other examples, lile the ALK/ELM4 fusion protein found in about 4-7% NSCLC patients, that has potential therpeutic impact as Dr. Arrieta showed yesterday; BRAF mutation in melanoma; and exon 11 c-Kit mutation in imatinib-responsive GIST patients.

In conclusion, PCR is a powerful tool for the detection of clinically relevant mutation in colon cancer, lung cancer, melanoma. And we can safely say that this technology is here to stay.

Thank you.