GEMCAD STUDY

Title | Shotgun Proteomics for Predicting Response to Anti-EGFR Therapy

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Study Synopsis
We propose a differential protein expression study based on label-free quantification to compare proteomics patterns between patients that achieve a radiological response or have a progression (RECIST criteria) during the anti-EGFR treatment (PULSE and POSHIBA Phase II Trials).

Objective: The main objective is to characterize the proteome of these clinical samples, trying to develop new predictive molecular tools to facilitate decision-making in the treatment of colorectal cancer.

Methods: We want to compare the proteomics patterns of 40 samples of each type: responders vs non-responders to anti-EGFR therapy. We will prepare protein extracts from FFPE biopsies and analyse their proteomic patterns by nano-LC-MS/MS experiments on a high-resolution mass spectrometer (Orbitrap Velos or similar equipment). For more information on technical details, see: Gámez-Pozo A et al. Shotgun proteomics of archival triple-negative breast cancer samples. Proteomics Clin Appl. 2013; 7: 283-291.

Hypothesis
Our hypothesis proposes that deep proteomics analyses allow characterizing the complete scenario of signaling pathways and biological processes altered as a result of the mutations produced in each tumor and can provide new predictive molecular tools for the treatment of colorectal cancer.

Primary Objective: The main objective of the research project is to develop a new proteomics signature able to identify those tumors that will respond to the chemotherapy/anti-EGFR combined therapy.

Secondary Objectives.
1. Determining if the proteomic fingerprint is related with the time to progression.
2. Determining if the proteomic fingerprint is related with survival.

Randomization | Not applicable

Scientific rationale
Survival of patients with metastatic colorectal cancer has improved over the last decade. In the late 90s, the median overall survival (OS) for patients with metastatic colon cancer treated with a regime based on 5-fluorouracil (5-FU) was only about 12 months (1). The addition of irinotecan and oxaliplatin increased overall survival (OS) to approximately 18 months (2). The advent of targeted therapy produced more increase in OS, which is close to 24-30 months in some studies (3).
During the last decade, a better understanding of the processes involved in the transformation of normal cells into tumor cells has led to the development of new drugs called targeted therapies. The term targeted therapy refers to drugs that are selectively directed against specific molecular pathways involved in tumorigenesis or tumor progression. To date, three targeted
agents: bevacizumab, cetuximab and panitumumab have been approved by the FDA and EMA for the treatment of colorectal cancer. Bevacizumab targets a ligand of vascular endothelial growth factor receptor, VEGFR, while cetuximab and panitumumab both target the epidermal growth factor receptor (EGFR). Other novel targeted therapies such as the antiangiogenic Aflibercept (4) or the multikine inhibitor Regorafenib (5), are now approved by the FDA in USA. Recent studies have revealed the existence of a molecular factor determining the response to treatments directed against EGFR - the absence of activating KRAS mutations in tumor cells - that has been proven as the best predictive marker of response to cetuximab and / or panitumumab as monotherapy or in combination with chemotherapy in colon cancer. Recent retrospective analyses of several large studies show that patients with tumors carrying KRAS mutation do not respond to cetuximab-based therapy or panitumumab. (6-10). However, the lack of KRAS mutations in tumors does not guarantee a response to EGFR inhibitors. Cetuximab and panitumumab are effective in only 10-20% of patients with an EGFR/RAS/RAF/MAPK unabridged pathway (11, 12).

Previous experience in colon cancer and other tumors shows that it is necessary to identify predictive markers of clinically significant response to targeted therapies. Small improvements in terms of progression-free survival (PFS) and increased toxicity reported in some studies do not justify the use of these drugs in an unselected population. Beyond the obvious clinical benefit to patients, it is clear that the identification of predictive markers will also help reduce the cost of cancer treatments (13, 14). It seems clear that the best way to unravel the mechanisms of response to anti-EGFR treatments is the global analysis of the signaling pathways initiated by EGFR. Indeed, the simultaneous analysis of the multiple molecular events involved in oncogenic signaling cascades initiated by EGFR can improve the predictive power of the biomarkers used individually (15).

Proteomics has situated at the forefront of biomedical research once the genomes of many organisms, including humans, have been sequenced. The term proteomics refers to the large-scale study of the proteins that make up a given cell, tissue or organism; that is, its proteome (16). Its importance lies in that it is precisely the proteins that ultimately define the role and control the functioning of cells, tissues and organisms (17).

Mass spectrometry has become a powerful tool to analyze whole biological systems. Recent technological advances allow us to identify thousands of proteins from tissue amounts compatible with clinical routine. This expanded coverage allows seeking molecular signatures underlying the biological processes associated with complex diseases. For example, thousands of mutations have been described associated with cancer, but the exact relationship between genomic variation and the resulting phenotype in each individual tumor remains a mystery. Deep proteomics analyses are necessary to try to characterize the complete scenario of signaling pathways and biological processes altered as a result of the mutations produced in each tumor.

We propose a differential protein expression study based on label-free quantification to compare proteomics patterns among patients enrolled in PULSE and POSHIBA Phase II Trials. The main objective of the proposed research project is to characterize the proteome of these clinical samples, trying to develop new predictive molecular tools to facilitate decision-making in the treatment of colorectal cancer. The clinical objectives are
correlation of biomarkers with time to progression, overall survival and response rate.

References:

Sample Size
It is assumed that QT/anti-EGFR combination will result in a 50% response rate (CR + PR) and, approximately, a 20% of progressions. Therefore, we will study the clinical and proteomics data of 40 patients from the PULSE study who have obtained response to treatment. These patients will be compared with the data of 40 patients who have progressed on first evaluation. The latter group will come from PULSE and POSHIBA studies indistinctly, (assuming that there will only be 15-20 progressions in PULSE).

Power for the biomarker study
N/A

Summary of Subject Eligibility Criteria
**Inclusion criteria**
40 patients included in the PULSE study with partial or complete radiological response at first evaluation and 40 patients who have progressed on first assessment and were included in the PULSE or POSHIBA studies.

**Exclusion criteria**
- Patients whose tumor tissue is not available for proteomics analyses.
- Patients in which response has not been evaluated.
- Patients whose response to treatment was stable disease.

Economic support
The promoter team.
Mass spectrometry analyses will be carried out through a partnership with the European Consortium PRIME-XS (www.primexs.eu).

Scheduled calendar
Timing of recruitment, follow-up, analysis and manuscript writing.
Most patients are already enrolled. At most, it should take six months to complete the required number of cases. After this, sample preparation, mass spectrometry experiments and data analyses should take an additional six months period. Finally, manuscript writing should require no more than three months.
Planned patients: 80
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