INTRODUCTION
The Epidermal Growth Factor Receptor (EGFR) signaling system is frequently unbalanced in non-small-cell lung cancer (NSCLC) and the ligand Epidermal Growth Factor (EGF) could be an attractive target. EGFR tyrosine kinase inhibitors (TKI) are a standard treatment in EGFR mutated tumors but resistance invariably appears due to various mechanisms, such as the secondary mutation p.T790M or the activation of alternative pathways (MET, AXL). We have assayed the antibodies raised by a “cancer vaccine” against human EGFR that block EGFR-EGFR interaction. This “cancer vaccine” has been tested successfully in an unselected population in a Phase III clinical trial.

OBJECTIVES
1) To study the effects of the anti-EGF antibodies, single agent and combined with Gefitinib or AZD9291, in the EGFR pathway, cell viability and cell cycle.
2) To assess if sera from immunized patients can inhibit the EGFR pathway.
3) To analyze the mechanism of action of anti-EGF antibodies and the combination with Gefitinib or AZD9291 in membrane receptors, downstream molecules and stem cell and apoptosis markers related to EGFR TKI resistance.

METHODS
Cell lines and antibodies: PC9 is a NSCLC cell line mutated in exon 19 of EGFR. Anti-EGF antibody was prepared in Bioven’s Pathway targeting Immunotherapy by immunizing rabbits.

Cell viability assays: Cells were plated in 96-well plates and incubated with the drugs and EGF for 72 h. Cell viability was assessed by the thiazolyl blue tetrazolium bromide (MTT) assay. Assays were performed in triplicates.

Western blot analyses: Cell lines were serum starved and treated for 2 or 24 h. All the antibodies were purchased from Cell Signaling Technology. Gefitinib was added at 0.25 µM and AZD9291 at 0.2 µM.

Cell Cycle: Cells were fixed in 70% cold ethanol and stained with propidium iodide and cell cycle analysis was made by flow cytometry.

RNA analysis: RNA expression was analyzed using quantitative retrotranscription PCR (Q-RT-PCR).

RESULTS

CONCLUSIONS

1) Anti EGF and sera from patients immunized with the “EGF cancer vaccine” inhibit EGFR signaling in an EGFR mutated NSCLC cell line model. The combination of anti-EGF antibodies and Gefitinib or AZD9291 increases the EGFR signaling inhibition.

2) Anti EGF antibodies inhibit the expression of proteins related to EGFR TKI resistance, including membrane receptors (AXL), downstream signaling molecules (Notch3) and stem cell markers (Bmi1 and Hes1), and increase PARP cleavage. The expression of AXL is controlled by EGFR through ERK1/2, and the downregulation of AXL by the Anti-EGF Abs.

3) Based on these preclinical results, a Phase II trial will be initiated to assess the effects of the “EGF cancer vaccine” first line in combination with Gefitinib in EGFR mutated NSCLC patients.