

# PHARMACODYNAMIC STUDY OF NIMOTUZUMAB, AN ANTI-EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) MONOCLONAL ANTIBODY, IN PATIENTS WITH UNRESECTABLE SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK: A SENDO FOUNDATION STUDY

Abstract #6070

Poster #14D

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## Abstract

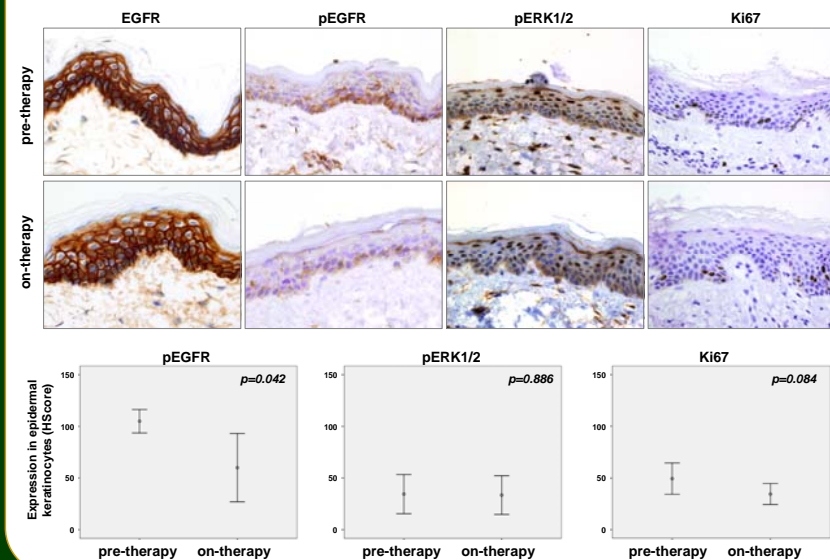
**Background:** Nimotuzumab is a humanized monoclonal antibody to Epidermal Growth Factor Receptor (EGFR) that has shown evidence of efficacy in patients with advanced solid tumors, notably without provoking skin rash. Although lack of rash might be a therapeutic advantage, evidence of biological activity of Nimotuzumab is needed. To this end, a pharmacodynamic (PD) study was performed.

**Methods:** Ten patients with advanced Squamous Cell Carcinoma of Head and Neck (SCCHN), unsuitable for chemo-radiotherapy, were enrolled in a single center phase Ib clinical trial. Patients received 8 weekly infusions of Nimotuzumab at 2 dose levels: 200mg and 400mg. The first Nimotuzumab infusion was administered 1 week before starting radiation while remaining doses were delivered concomitantly with irradiation. Paired biopsies were taken from skin and primary tumors, before (pre-therapy) and 1 week (on-single agent therapy) after first infusion. A PD study by immunohistochemistry was conducted to assay the effects of Nimotuzumab on EGFR -total and phosphorylated (p)- and its signaling pathways ERK and AKT (total and p) as well as proliferation (Ki67).

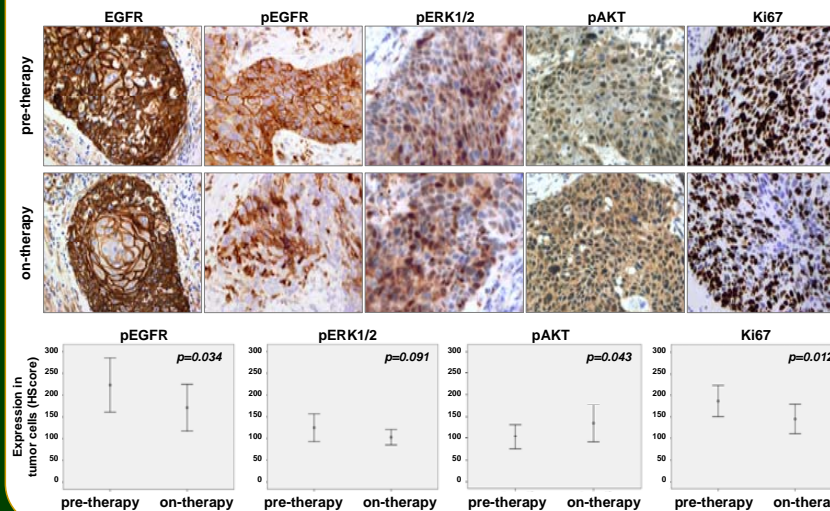
**Results:** Nimotuzumab was well tolerated and there was no evidence of skin rash in any of the treated patients. Objective response was achieved in 80% of patients (2 CR, 6 PR). Median survival time was 7.2 months. PD showed inhibition of p-EGFR in both skin and tumor (Wilcoxon test,  $p=0.042$  skin,  $p=0.034$  tumor). Inhibition of downstream pathways was suggested by a trend in the decrease of p-ERK ( $p=0.091$ ) and a significant reduction in proliferation ( $p=0.012$ ). Upregulation of p-AKT was observed in tumor ( $p=0.043$ ) but not in skin, similarly to what has been reported for other anti-EGFR agents. Characteristic lymphocytic infiltrates, folliculitis or perifolliculitis induced by other EGFR inhibitors were not observed. No associations were found between doses or response and PD effects.

**Conclusions:** These data show that after a short period of exposure, Nimotuzumab as a single agent inhibited EGFR phosphorylation and this was accompanied by a trend towards molecular downstream effects consistent with the expected biological effects of EGFR targeting. The absence of inflammatory skin reaction might be linked to the lack of skin toxicity by Nimotuzumab. The response rate observed with Nimotuzumab and radiation is promising.

## Results: Skin pharmacodynamic analysis



## Results: Tumor pharmacodynamic analysis



## Patient characteristics

Main characteristic of the patients							
Patients	Sex	Age	Performance Status	TNM	Stage	Site of primary tumor	
1	M	70	1	T3N1M0	III	Base of tongue	
2	M	64	0	T3N2M0	IVa	Tonsil, soft palate	
3	M	63	1	T3N2aM0	IVa	Hard palate, soft palate, tonsil, retromolar trigone	
4	F	62	0	T3N2bM0	IVa	Anterior tongue, base of tongue	
5	M	63	0	T3N2aM0	IVa	Hard palate, soft palate, tonsil, retromolar trigone, alveolar ridge	
6	M	53	0	T3N0M0	III	Retromolar trigone, tonsil, base of tongue	
7	M	55	1	T2N2cM0	IVa	Posterior pharyngeal wall	
8	M	61	0	T3N0M0	III	Tonsil	
9	F	73	1	T4N1M0	IVa	Anterior tongue, base of tongue, soft palate, tonsil	
10	M	66	0	T3N2cM0	IVa	Tonsil, anterior tongue, base of tongue	

## Treatment outcomes

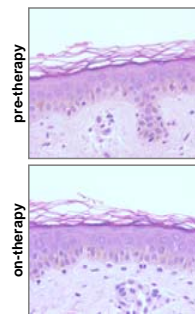
Patients	I+R3 doses (mg)	Number of doses	RT doses (Gy)	Treatment outcomes					
				Complete treatment	Best Response	Duration of response (weeks)	2nd line treatment	Status at last observation	Survival time (months)
1	200	8	66	Yes	CR	27	MTX	Death	16.1
2	200	8	66	Yes	PR	19	No	Death	7.2
3	200	8	66	Yes	PR	22	MTX	Death	10
4	200	7	54	No	uPR	11	No	Death	4.8
5	200	8	66	Yes	PR	12	MTX	Death	11.8
6	400	8	66	Yes	PR	7	MTX	Death	7.9
7	400	1	66	No	PR	10	No	Death	5.8
8	400	8	66	Yes	CR	109	No	Alive	29.7
9	400	7	56	No	SD	14	No	Death	3.4
10	400	6	38	No	uPR	14	No	Death	4

CR=complete response, PR=partial response, uPR=unconfirmed partial response, SD=stable disease, PD=Progression disease, MTX weekly metotrexate  
 Four patients interrupted the planned treatment:  
 Patient 04: alteration the treatment  
 Patient 07: developed grade 2 allergic reaction (urticaria) after first infusion of Nimotuzumab  
 Patient 09: discontinued because negative of performance status  
 Patient 10: discontinued treatment after severe adverse event (heart failure)

## Pharmacodynamic analysis

To assess the impact of Nimotuzumab on signalling through the EGFR pathway, skin punch biopsy specimens and surgical tumor samples were obtained from 10 patients who provided additional informed consent. Biopsies were obtained before and after 7 days of treatment. The following pharmacodynamic markers were assessed with appropriate antibodies: EGFR (mouse MAb clone 2-18C9, DAKO, Carpinteria, CA), phosphorylated EGFR (pEGFR) (mouse MAb clone 74, Chemicon, Temecula, CA), the downstream signaling markers phosphorylated mitogen-activated protein kinase (pERK1/2) (rabbit polyclonal phospho-p44/42 MAPK Thr202/Tyr204 antibody, Cell Signaling Technology, Beverly, MA) and phosphorylated protein kinase AKT (rabbit polyclonal phospho-AKT Ser473 antibody, Cell Signaling Technology, Beverly, MA) and the proliferation marker Ki67 (mouse MAb clone MIB1, DAKO, Carpinteria, CA). All markers were evaluated by immunohistochemistry, to determine both the percentage of target cells stained by each marker and the staining intensity. Immunostaining was performed using 4  $\mu$ m tissue sections on positively charged glass slides, as previously described (Rojo, F. et al, J Clin Oncol, 2006).

Basal expression levels of EGFR, pEGFR, pERK1/2, pAKT and Ki67 were analyzed in skin and tumor biopsies and compared with expression levels on Day 7 following Nimotuzumab treatment. In skin, Nimotuzumab inhibited the phosphorylation of EGFR and decreased the expression of Ki67 in the keratinocytes of the epidermis. Expression levels of pERK1/2 were not affected by Nimotuzumab treatment. Interestingly, Nimotuzumab did not result in previously described presence of perivascular inflammatory lymphocytic infiltrates in superficial dermis using EGFR inhibitors (see right panel). This relevant feature would be explained by the low antigenicity of the antibody or, alternatively by the short period of exposition to the drug in the study. In tumor, Nimotuzumab inhibited the activated EGFR and proliferation in tumor cells and reduced the phosphorylation of signalling pathway ERK.



## Conclusions

1. Nimotuzumab as a single agent inhibited EGFR phosphorylation both in skin and tumor, and this was accompanied by a trend towards molecular downstream effects consistent with the expected biological effects of EGFR targeting.
2. The response rate observed with Nimotuzumab and radiation in SCCHN is promising.
3. Nimotuzumab would be a window of opportunity in SCCHN before adding radiation therapy.