Improved response by co-targeting EGFR/EGFRvIII and c-Src

Mette Villingshøj,[1] Peter Andersen,[1] Marie-Thérése Stockhausen,[1] Lone Ottesen[2] and Hans S. Poulsen[1]

[1]Department of Radiation Biology, Section 6321, Finsen Center, Copenhagen University Hospital, Copenhagen, Denmark

[2]Medical Department, AstraZeneca, Roskildevej 22, Albertslund DK-2620, Denmark

e-mail: peter.andersen@rh.regionh.dk

www.radiationbiology.dk

Background

- Overexpression of the epidermal growth factor receptor (EGFR) and the EGFR variant EGFRvIII is often associated with human cancer
- Recent results have demonstrated that the non-receptor tyrosine kinase c-Src is involved in EGFR-mediated signaling and works synergistically with EGFR in tumor proliferation, invasion and metastasis.
- We hypothesized that co-treatment with gefitinib and the novel Abl/c-Src inhibitor AZD0530 would improve the response in human cancer cells

Aim

To evaluate the effects of gefitinib and AZD0530 in human cancer cell lines

Materials and Methods

- **Cell lines:** The human head and neck carcinoma cell line HN5, the human skin carcinoma cell line A431 - both over-expressing EGFR, and the murine fibroblast NR6M – expressing EGFRvIII
- **Immunoblot:** Protein lysates were resolved by SDS-PAGE, electroblotted onto nitro-cellulose membranes, incubated with anti-bodies and visualized by ECL
- **MTT assay:** Cells were plated in 96 wells, serum-starved for 24 hours and treated with various concentrations of AZD0530, gefitinib and/or EGF, and incubated for 72 hours prior to MTT addition
- **Wound healing assay:** Confluent cells were wounded, and the remaining cells treated with various concentrations of AZD0530, gefitinib and/or EGF. Migration of cells was observed at 0, 24, 48 and 72 hours. Random selected images were acquired with a phase contrast microscope (Nikon)

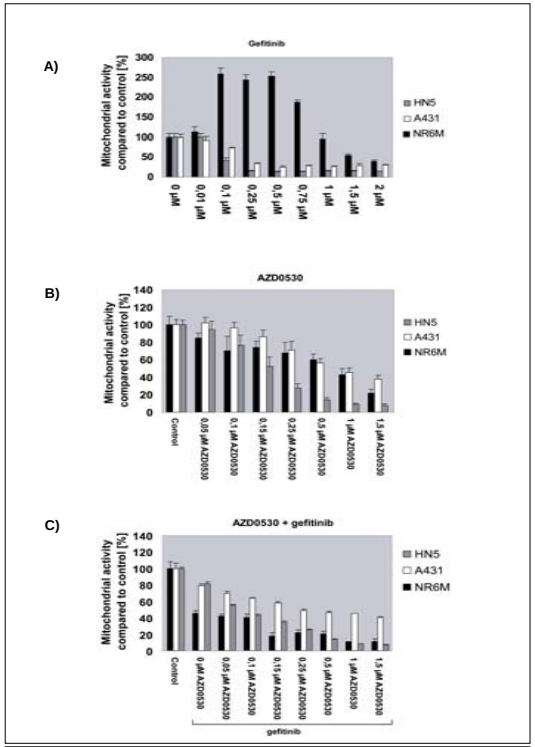

Fig. 1: MTT assays: A) Cells were treated with the indicated gefitinib concentrations for 72 hours. B) Cells were treated with the indicated AZD0530 concentrations for 72 hours. C) Cells were treated with the indicated AZD0530 concentrations for 72 hours in presence of gefitinib (gefitinib concentrations: HN5 – 0,025 µM, A431 - 0,1 µM, NR6M – 1,9 µM).

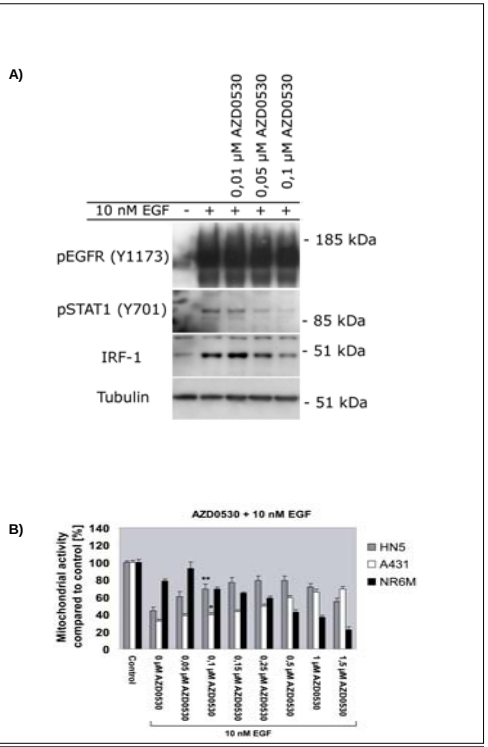

Fig. 2: A) Western blot analysis showing phosphorylation status of EGFR and STAT1 along with expression of IRF-1 in HN5 cells treated with 10 nM EGF alone or in combination with AZD0530 for one hour (similar results were observed in A431 cells). B) MTT assay: Cells were treated with the indicated AZD0530 concentrations for 72 hours in presence of 10 nM EGF. * ($P<0.05$), ** ($P<0.001$).

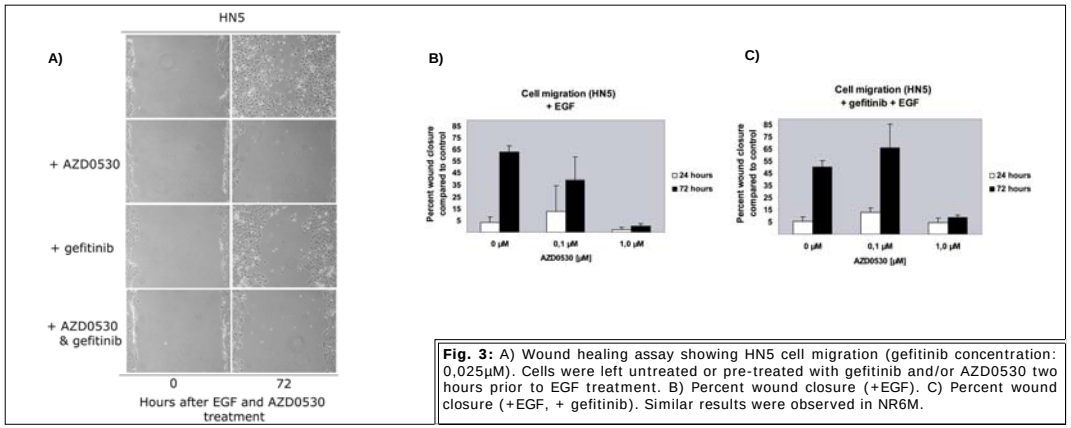

Fig. 3: A) Wound healing assay showing HN5 cell migration (gefitinib concentration: 0,025µM). Cells were left untreated or pre-treated with gefitinib and/or AZD0530 two hours prior to EGF treatment. B) Percent wound closure (+EGF). C) Percent wound closure (+EGF, + gefitinib). Similar results were observed in NR6M.

Results

- Gefitinib inhibited cell viability in EGFR expressing cells, but increased proliferation in EGFRvIII expressing cells **(Fig. 1A)**
- AZD0530 effectively inhibited cell viability in all cell lines **(Fig. 1B)**

	IC_{50} (µM)	
Cell line	Gefitinib	AZD0530
HN5	0,03	0,17
A431	0,12	0,75
NR6M	1,9	0,75

- Co-treatment with AZD0530 and gefitinib resulted in an additive inhibition of cell viability **(Fig. 1C)**
- AZD0530 treatment was associated with inhibition of Src regulated gene expression and activation (pSTAT1 and IRF-1), and did not inhibit EGFR activation **(Fig. 2A)**
- AZD0530 treatment reduced EGF-mediated growth inhibition **(Fig. 2B)**
- AZD0530 effectively inhibited cell migration **(Fig. 3A-C)**

Conclusions

- **Low concentrations of gefitinib increase proliferation of EGFRvIII-expressing cells**
- **In contrast, AZD0530 effectively reduces viability of both EGFR and EGFRvIII-expressing cells**
- **Co-treatment with AZD0530 and gefitinib results in an additive reduction of cell viability**
- **AZD0530 effectively inhibits Src-dependent gene expression and reduces the growth inhibitory effects of high EGF concentrations (10 nM)**
- **AZD0530 is a potent inhibitor of cell migration**