Targeting resistance in EGFR-mutant non-small cell lung cancer (NSCLC): preclinical evidence supporting the combination of EGFR tyrosine kinase inhibitors (TKIs) AZD9291 and gefitinib with molecularly targeted agents and immunotherapeutics

[1]Darren Cross, [2]Catherine Meador, [1]Cath Eberlein, [2]Paula Spitzler, [2]Eiki Ichihara, [3]Evan Barry, [4]Edmund Poon, [4]Arthur Lewis, [1]Susan Ashton, [1]Martine Mellor, [1]Paul Smith, [3]Alwin Schuller, [3]Melanie Frigault, [2]Christine Lovly, [4]Robert Wilkinson, [4]Ross Stewart, [3]Celina D'Cruz, [2]William Pao, [1]Philip Jewsbury

[1]AstraZeneca, Alderley Park, Macclesfield, UK; [2]Vanderbilt-Ingram Cancer Center, Nashville, TN, USA; [3]AstraZeneca, Gatehouse Park, Waltham, MA, USA; [4]MedImmune Ltd, Granta Park, Cambridge, UK

Poster 466P

Introduction

- AZD9291 is an oral, potent, irreversible epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) selective for sensitising (EGFRm+) and T790M resistance mutations over wild-type EGFR.[1]
- Despite effectiveness of current EGFR-TKIs as first-line treatment for patients with advanced EGFRm+ non-small cell lung cancer (NSCLC), most patients progress after 9–14 months.[2,3]
- Data from a Phase I dose escalation/expansion study (AURA; NCT01802632) have shown that AZD9291 has clinical activity in patients with EGFRm+/ T790M+ advanced NSCLC who have progressed after EGFR-TKI treatment.[4] However, experience with targeted therapies suggests that acquired resistance to these agents may emerge.
- Combining EGFR-TKIs with molecularly targeted agents, and immunotherapeutics such as checkpoint inhibitors, has the potential to delay emergence of resistance or address acquired resistance in later lines.
- Here we report emerging preclinical evidence supporting clinical investigation of combining EGFR-TKIs gefitinib and AZD9291 with specific molecularly targeted agents.

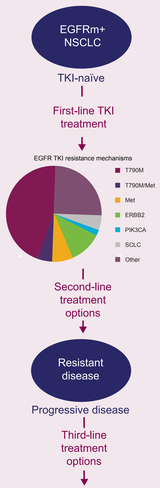


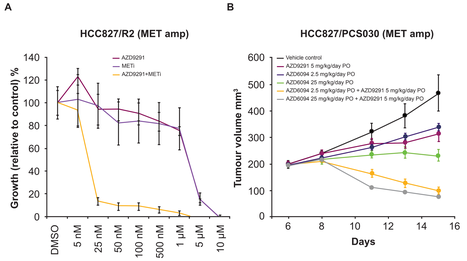
Table 1. Agents available for combination studies

Agent	Properties
AZD9291	Irreversible TKI targeting EGFRm+ and T790M
Gefitinib	Reversible TKI targeting EGFRm+ and wild-type EGFR
AZD6094 (HMPL-504)	Reversible small molecule inhibitor targeting MET
Selumetinib (AZD6244, ARRY-142886)	Reversible small molecule inhibitor targeting MEK1/2
MEDI4736	Biological antibody targeting PD-L1
Tremelimumab	Biological antibody targeting CTLA-4

Addressing MET driven acquired TKI resistance

- MET amplification has been associated with acquired resistance to early generation EGFR-TKIs in 10–15% of patient cases.[5]
- Targeting MET inhibition in combination with TKI could provide an effective strategy to increase benefit in this subset of patients.[6]
- AZD6094 (HMPL-504), a small molecule MET inhibitor, is being investigated in clinical studies.
- Preclinical studies are being carried out to better understand potential clinical application of this combination.

Figure 1. Combination therapies of AZD9291 and MET inhibitor are required to overcome resistance in EGFRm+ EGFR-TKI-resistant tumours that have acquired MET gene amplification

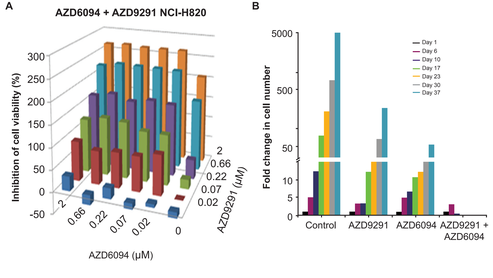


A) HCC827/R2 and B) HCC827/PCS030 cell line derivatives with acquired resistance to erlotinib through high MET gene amplification. A) Treatment with AZD9291 and a small molecule MET inhibitor in combination was necessary to achieve growth inhibition *in vitro*. B) Treatment with AZD9291and AZD6094 in combination was necessary to achieve significant tumour shrinkage *in vivo*. PO, orally

These data demonstrate that combination of an EGFR-TKI such as AZD9291 or gefitinib with a MET inhibitor such as AZD6094 is necessary and sufficient to address acquired resistance due to MET gene amplification.

Delaying emergence of MET driven TKI resistance

Figure 2. Treatment with AZD9291 combined with AZD6094 delays time to resistance of a EGFRm+/T790M and MET over-expression (CN~4) cell line (NCI-H820) *in vitro*



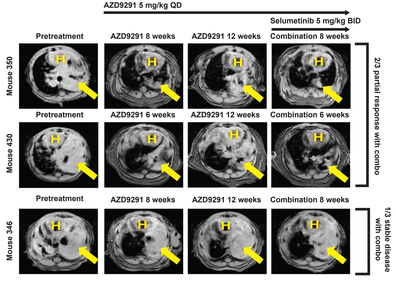
NCI-H820 cell line (EGFR ex19del/T790M with increased MET copy number) was treated with AZD9291 and AZD6094 alone or in combination. A) Cells were treated for 5 days with AZD9291 and AZD6094 in a dose response combination matrix, and showed that the combination of AZD9291 and AZD6094 had only moderate synergy on inhibition. In contrast, B) after chronic treatment with AZD9291 (160 nM) and AZD6094 (250 nM) alone or in combination for 37 days (confluent cells were harvested, counted and reseeded at the original seeding density to track increase in total cell number over time). Long-term treatment with AZD9291 and AZD6094 in combination was required to delay emergence of resistant cell lines

These data support the combination of an EGFR-TKI such as AZD9291 with a MET inhibitor such as AZD6094 to delay MET induced resistance in settings of co-existing T790M and MET over-expression.

Addressing acquired resistance to TKIs by inhibiting MEK/ERK pathway with selumetinib

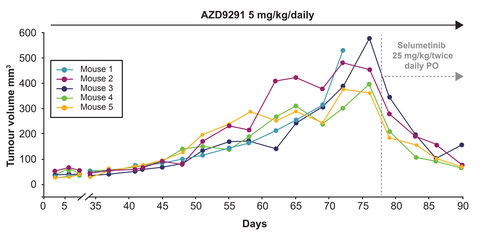
- MEK/ERK is a key signalling pathway downstream of mutant EGFR.
- Increasing evidence suggests that switching dependence to the MEK pathway independently of EGFR could be a frequent mechanism of TKI resistance.[7]
- Preclinical studies are ongoing to explore the role and molecular mechanisms of MEK/ERK pathway switching in EGFR-TKI resistance, which will help inform the potential application for combining EGFR-TKIs with the MEK1/2 inhibitor selumetinib.

Figure 3. Treatment with a combination of AZD9291 and selumetinib can restore tumour growth inhibition following progression on AZD9291 in a T790M transgenic tumour model *in vivo*.



L858R/T790M tumours became resistant to 5 mg/kg/day of AZD9291 after about 12 weeks. Mice were then treated with a combination of AZD9291 and selumetinib. 2/3 mice showed subsequent partial response (PR) of shrinkage and one mouse showed stable disease (SD). Arrow denotes tumour. A dose of 5 mg/kg/day is equivalent to clinically relevant dose of 20 mg QD. BID, twice daily; H, heart; QD, once daily

Figure 4. Treatment with a combination of AZD9291 and selumetinib can restore tumour growth inhibition in a PC9/AZD9291-resistant xenograft model (NRAS 1.3 CN gain, MAPK1 1.5 CN gain) *in vivo*

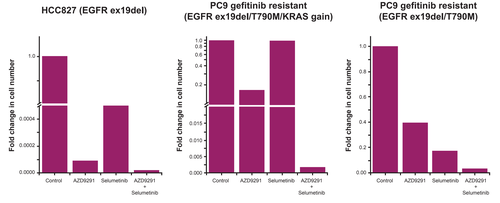


PC9 population developed to have acquired resistance to AZD9291 *in vitro* were implanted into NODSCID mice under constant selective AZD9291 dosing pressure. Preliminary results in a growth curve setting show a significant reduction in tumour volume when selumetinib was combined with AZD9291 in all animals treated with the combination therapy

These data support the combination of an EGFR-TKI such as AZD9291 or gefitinib with a MEK inhibitor such as selumetinib to address acquired resistance due to dependence on MEK/ERK pathway.

Delaying emergence of MEK driven TKI resistance

Figure 5. Treatment with a combination of AZD9291 and selumetinib can delay time to resistance across multiple cell backgrounds *in vitro* in long-term treated (~20 days) models

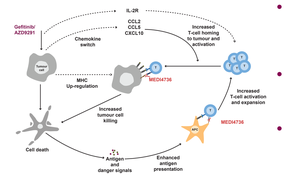


Multiple cell populations representing different genetic backgrounds were treated with AZD9291 (160 nM) and selumetinib (100 nM) alone or in combination for ~20 days as described in Figure 2B. The data showed that the combination was more effective at delaying resistance across all models with different molecular contexts

These data support combining an EGFR-TKI such as AZD9291 or gefitinib and a MEK1/2 inhibitor such as selumetinib to delay acquisition of resistance in settings where MEK/ERK signalling is important for driving TKI resistance.

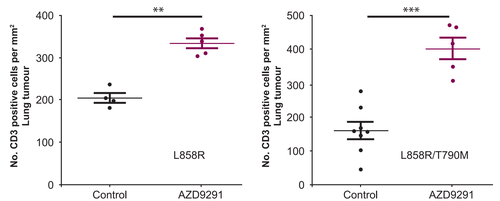
Prolonging treatment benefit through combining TKIs with immunotherapies

Figure 6. Combining EGFR-TKI with immunotherapies such as MEDI4736 (anti-PD-L1) could enhance the anti-tumour immune response and extend treatment benefit



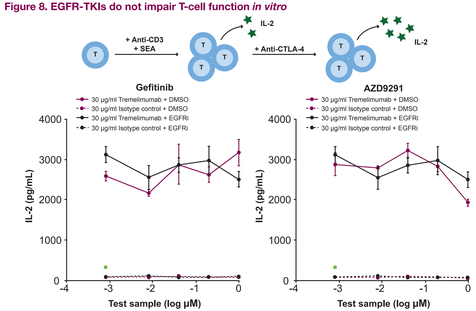
- Inhibition of EGFR may promote anti-tumour immunity via multiple mechanisms including upregulation of MHC and release of chemokines that attract T-cells
- Tumour cell death induced by EGFR-TKI has the potential to prime the immune system through antigen release
- Preclinical studies are ongoing to better understand the opportunity for immunotherapy combinations

Figure 7. Treatment with AZD9291 drives increased T-cell infiltration into NSCLC tumours in mice



Transgenic mice bearing L858R or L858R/T790M lung tumours were treated with 5 mg/kg/day of AZD9291 for 2 weeks. Significant tumour regression was accompanied by an increase in T-cell infiltration (as measured by CD3 immunohistochemistry).

Figure 8. EGFR-TKIs do not impair T-cell function *in vitro*



Tremelimumab-stimulated secretion of IL-2 from primary human peripheral blood mononuclear cells *in vitro* was not affected by treatment with either gefitinib or AZD9291. IL, interleukin

This suggests EGFR-TKI does not have direct effects on T-cell function.

Conclusions

- Acquired resistance to EGFR-TKIs will limit treatment benefit in most cases. Combining EGFR-TKIs with molecularly targeted agents, and immunotherapeutics such as checkpoint inhibitors, has the potential to address acquired resistance or prevent emergence of resistance across lines of therapy.
- Emerging preclinical and clinical data suggest targeting MET, e.g. with AZD6094, in combination with an EGFR-TKI could address or delay resistance associated with dependence on MET signalling.
- Similarly, ongoing preclinical data suggest targeting MEK pathway, e.g. with selumetinib, in combination with an EGFR-TKI could address or delay resistance associated with dependence on MEK/ERK signalling.
- Other therapeutic strategies are also being explored such as combining with immunotherapy checkpoint inhibitors (e.g. MEDI4736) or anti-angiogenic agents.
- Therefore further preclinical and clinical evaluation is warranted. A multi-targeted agent clinical trial has been initiated to set the combination doses for several combinations (NCT02143466).

Acknowledgements

We thank PRECOS Ltd, a Crown Bioscience Company, Loughborough, UK, Rajendra Kumari and Andrew McKenzie for supporting the study shown in Figure 1B, and also Oncotest GmbH, Freiburg, Germany and Oliver Rath for supporting the study shown in Figure 4.

The study was sponsored by AstraZeneca. We thank Claire Shewbridge and Karen Smith, from iMed Comms, who provided editing assistance funded by AstraZeneca.

References

1. Cross et al. Cancer Discov 2014;4:1046–1061.
2. Mok et al. N Engl J Med 2009;361:947–957.
3. Rosell et al. Lancet Oncol 2012;13:239–246.
4. Jänne et al. J Clin Oncol 2014;32(Suppl): abstract 8009.
5. Engelman et al. Science 2007;316:1039–1043.
6. Wu et al. J Clin Oncol 2014;32(Suppl): abstract 8017.
7. Ercan et al. Cancer Discovery 2012;2:934–947.

Poster presented at: European Society for Medical Oncology 2014 Congress, 26–30 September, Madrid, Spain; Abstract #7054