

**ESMO Translational Research Fellowship**  
(October 2013-October 2015)

1. **Phase II multicenter proof of concept study of AZD4547 in FGFR amplified tumours**
2. **A retrospective translational study assessing the FGFR2 status of cholangiocarcinoma patients (Ongoing)**

**Noelia Tarazona**

**FINAL REPORT**

**Host Institute:** The Royal Marsden Hospital, London (United Kingdom)

**Mentor:** Prof David Cunningham/Dr Nicholas Turner

**Project title:** Phase II multicenter proof of concept study of AZD4547 in FGFR amplified tumours

**Home Institute:** Hospital Clínico Universitario de Valencia, Spain

***Rationale and Aim***

**1)** There are approximately 1.5 million new cases and over 1.1 million deaths from gastro-oesophageal cancer per year globally. The majority (60% to 70%) of patients with gastroesophageal cancer present with unresectable locally advanced or metastatic disease and more than half of those initially treated with curative intent will develop incurable recurrent locoregional or metastatic disease. TOGA study provided proof of concept for the successful use of targeted therapy in advanced oesophagogastric cancer and has driven investigators to examine other potential therapeutic targets for this disease including the *EGFR*, *MET*, *PI3K/MTOR* and *FGFR* pathways, the last of which is the focus of this grant application. There is strong preclinical evidence for using *FGFR2* inhibition as a therapeutic target in *FGFR2* amplified gastric cancers. The Royal Marsden Hospital Foundation Trust (RMH) in conjunction with the Institute of Cancer Research (ICR) are currently conducting a phase II non randomised study assessing the efficacy of AZD4547 in patients with FGFR amplified cancers.

**RESEARCH PROPOSAL:**

- A. Retrospective evaluation of prognostic and predictive effects of FGFR amplification in patients enrolled in MAGIC trial--> **it was not possible because of low-quality DNA.**
- B. Assessment of translational endpoints for FGFR study (plasma DNA analysis).  
Primary endpoint: objective confirmed response rate in each tumour group

The translational endpoint assessment of the ongoing FGFR trial (Part B) will provide essential information on the pharmacodynamic activity of this novel agent. These data may influence future trial designs for this drug

not only for patients with *FGFR* amplified gastroesophageal cancer but also for those with breast and lung malignancies.

- The study originally consisted of three independent tumour cohorts (*FGFR1* amplified breast and squamous NSCLC and *FGFR2* amplified gastroesophageal cancer), however due to poor accrual the lung cohort was closed to further recruitment.

- Treatment consisted of AZD4547 80mg twice daily (initially on an intermittent schedule of two weeks on, one week off which was subsequently amended to continuous dosing).

- One or more responses were required in the initial 9 patients in each cohort, to recruit a total of 17 patients. Three of more patients were required to conclude that cohort had sufficient efficacy for further study.

2) I extended my ESMO Fellowship for one more year because I had to finish the original project and I participated in another novel project (***FGFR2* gene fusions in cholangiocarcinoma: a novel therapeutic target**) but this project is ongoing and I still do not have results. Compelling evidence indicates that aberrant FGF signaling is involved in the pathogenesis of many malignancies. In fact, a growing body of research indicates that the inhibition of the FGF pathway may present an effective therapeutic option for cancer.

#### **Primary endpoint:**

- The proportion of cholangiocarcinoma patients in whom an *FGFR* translocations was detected (by FISH or ddPCR)

#### **Secondary endpoints:**

- The concordance of *FGFR* fusion results obtained from a ddPCR assay compared to the Zytovision FISH probe.

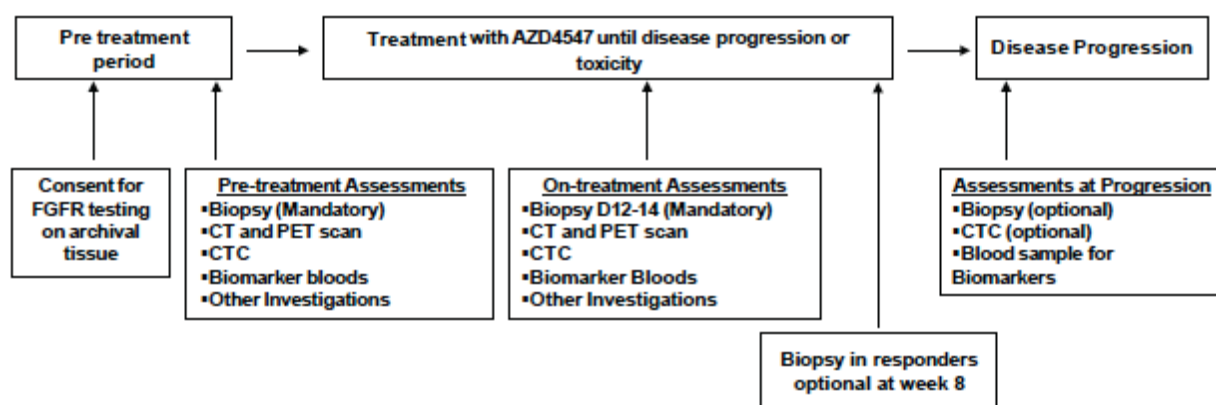
- Correlation of clinical baseline and outcome data (PFS, OS and Response) between patients with and without an *FGFR* fusion.

### **Experimental design**

#### **1. Assessment of translational endpoints for *FGFR* study**

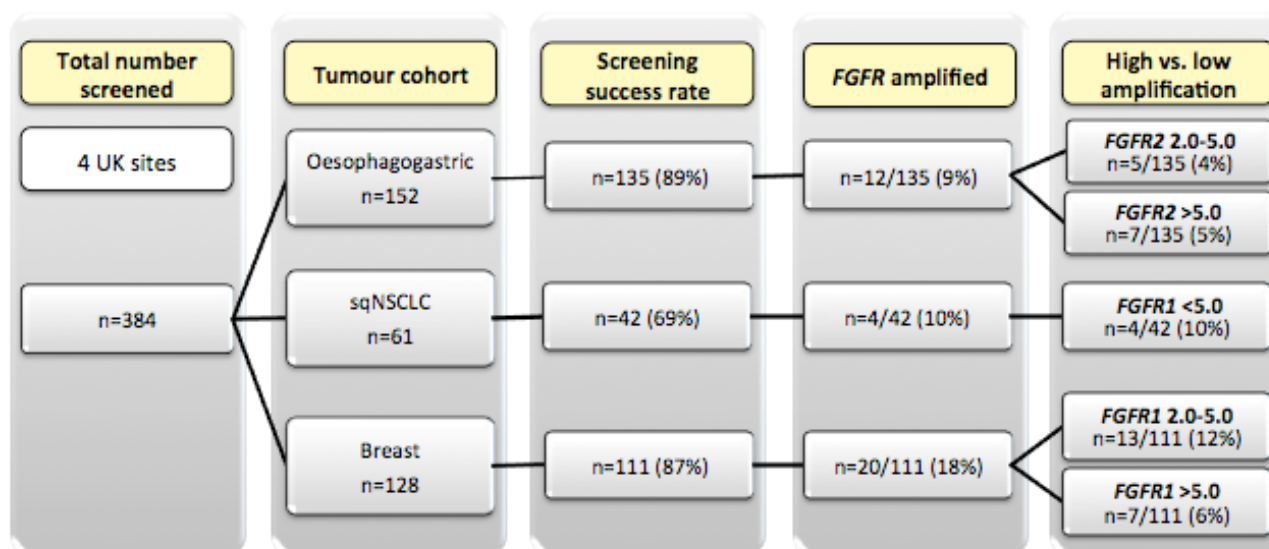
All patients entering the *FGFR* study undergo mandatory tumour biopsy on day 0 and day 14, with an optional biopsy on tumour progression. Archival tissue for patients entering the study was also requested from participating institutions. I assisted in the assessment of the endpoints of the study working with existing laboratory staff.

We assessed the activity of the FGFR selective inhibitor AZD4547 in FGFR1 amplified breast cancer and FGFR2 amplified gastro-oesophageal cancer. We screened 341 patients with advanced cancer by fluorescent in situ hybridization (FISH) for the presence of gene amplification, identifying FGFR1 amplification in 18% advanced estrogen receptor positive breast cancer and FGFR2 amplification in 9.0% advanced gastro-oesophageal cancer. Eight patients with FGFR1 amplified breast cancer and nine with FGFR2 amplified gastrooesophageal cancer were treated with AZD4547 in a translational clinical trial.



Schematic showing trial design of the FGFR trial (EudraCT No.:2011-003718-18) a phase II, open label, non randomised study of AZD4547.

#### FGFR Study Screening Results (September 2012 - June 2014)



## 2. Cholangiocarcinoma project

This project is a retrospective translational research study in patients with cholangiocarcinoma to be performed at RMH/ICR in collaboration with the UCL BRC.

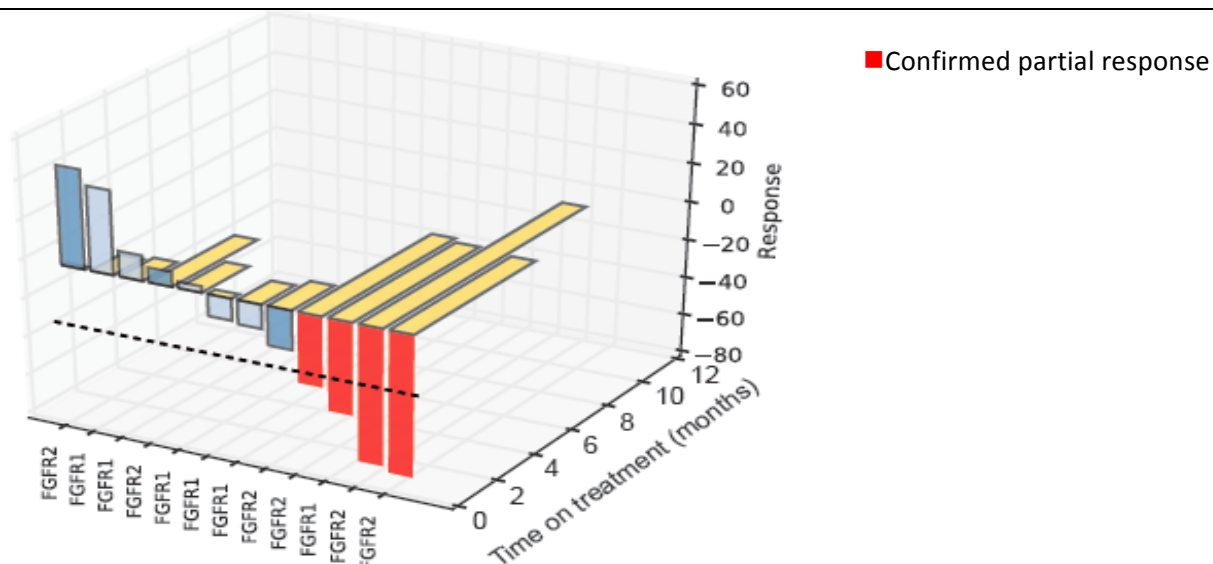
Tissue is available from approximately 170 cholangiocarcinoma patients at The Royal Marsden, and from 275 cholangiocarcinoma patients at University College London (collaborator Dr John Bridgewater). We plan to assess FGFR2 fusions in cholangiocarcinoma tissue using the Zytovision breakpart FISH probe. This work will be undertaken under the supervision of Dr David Gonzalez de Castro in our Centre for Molecular Pathology who has significant experience in FISH including testing for translocations/fusions clinically (such as ALK in NSCLC). Concurrently with the FISH work we propose to validate these FGFR2 fusions by ddPCR using fusion-specific primers. This work is being supervised by Dr Nicholas Turner and Alex Pearson from the Institute Cancer Research who have extensive experience in FGFR research and currently run the translational component of our prospective FGFR trial. Prior to assessment of the FGFR status of the archival cholangiocarcinoma tissue we plan to assess the accuracy of the FISH probe and ddPCR methodology in FGFR2 fusion positive cell lines which have been documented in the literature (SNU16 gastric and MCF7 breast cancer cell ones). In addition, we plan to test some translocations + cell lines. Following assessment of FGFR2 fusion status of the cholangiocarcinoma patients, we will correlate these molecular results with patient and tumour characteristics, chemotherapy response and overall survival.

## Results, Conclusions and Future Perspectives

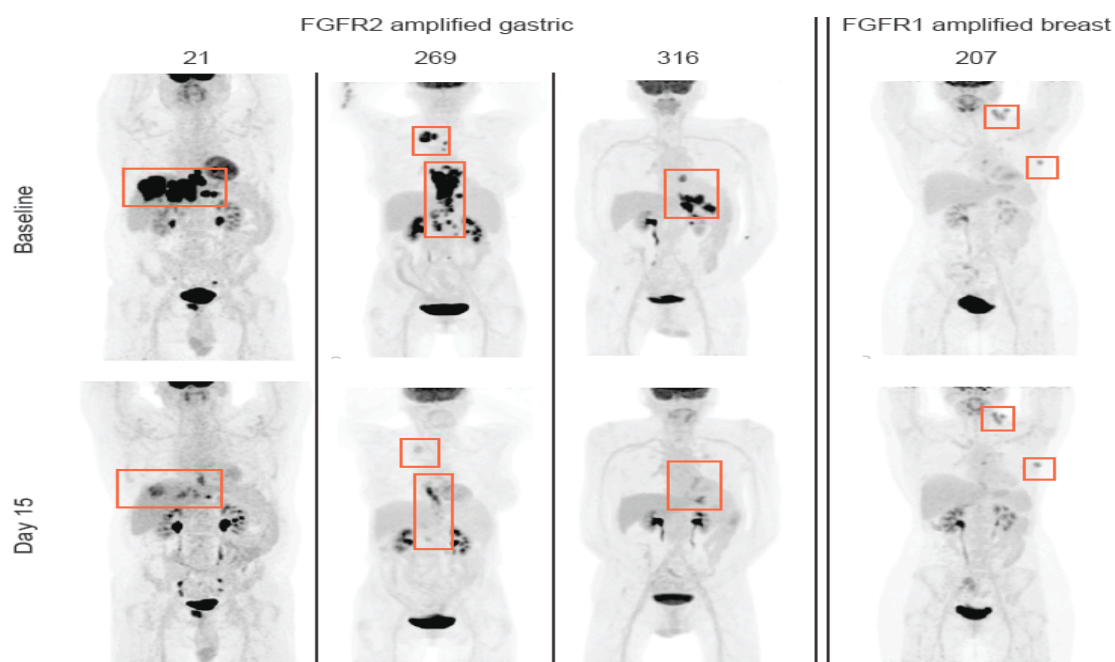
### Phase II multicenter proof of concept study of AZD4547 in FGFR amplified tumours

#### **FGFR2 amplified cancers have a high response rate to AZD4547**

One FGFR1 amplified (12.5% response rate) and three FGFR2 amplified (33% response rate) patients had confirmed responses to AZD4547, reaching the predefined criteria for efficacy in the FGFR2 amplified cohort. The responses were durable with a median progression free survival of responding patients of 6.6 months (range 6.2-10.5 months).

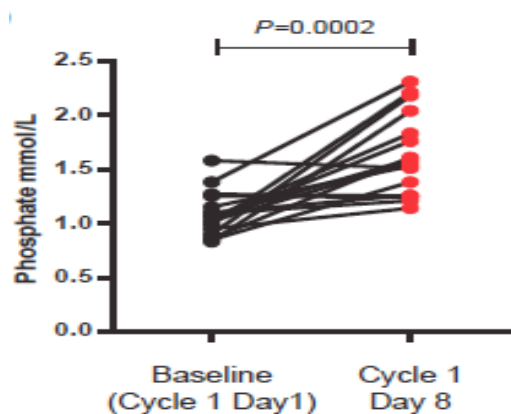


Comparison of 18F-fludeoxyglucose positron emission tomography (FDG-PET) in responding patients between baseline and day 15 demonstrated a substantial reduction in glucose uptake in all responding FGFR2 amplified gastric cancers, although there was no change in the FGFR1 amplified breast cancer.



The FDG-PET responses were maintained at 8 weeks. Two additional patients with FGFR2 amplified gastric

cancer had a response in day 15 FDG-PET but did not have a confirmed response by RECIST criteria (Patients 135 and 99). Serum phosphate was elevated from baseline in the majority of patients on the study ( $P=0.0002$  Wilcoxon matched pairs-signed rank test), as a pharmacodynamic marker of interrupting FGF23 signalling by FGFR inhibition.



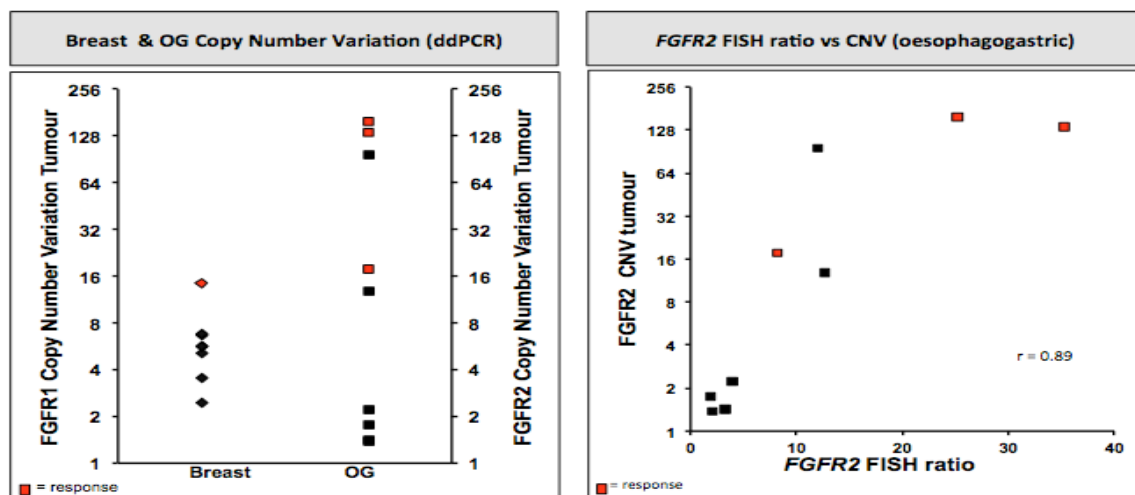
There was no correlation of response to change in phosphate level. Both a continuous and intermittent schedule of AZD4547 were used in the trial (methods). Of the responders, patient 21 and 207 were treated on the intermittent schedule, and patients 269 and patient 316 received continuous treatment.

#### **High-level clonal FGFR2 amplification is a therapeutic target for selective FGFR inhibitors**

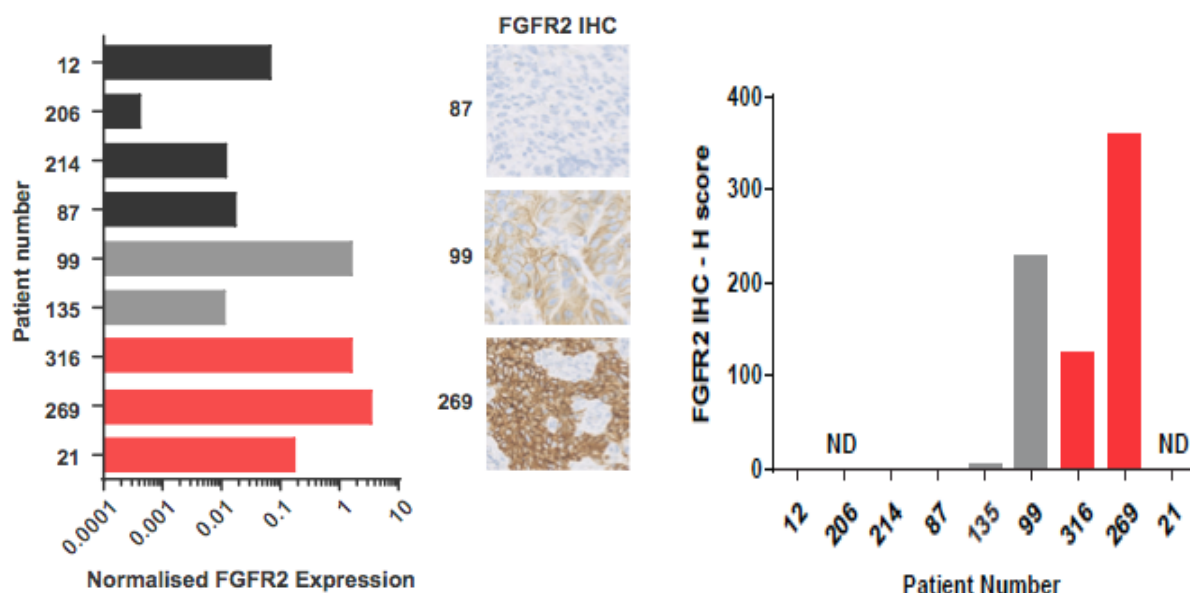
We investigated the pathological basis of response in FGFR amplified cancers, the relatively high response rate of FGFR2 amplified gastric cancers compared to FGFR1 amplified breast cancer, with FDG-PET reductions only in FGFR2 amplified cancers. We assessed relative FGFR copy number by digital PCR in baseline tumour biopsies of patients treated in the clinical trial.

Cancers with high copy number (high-level) FGFR amplification were more likely to respond to AZD4547 ( $P=0.0026$  Mann-Whitney U Test), with response only observed in cancers with high-level amplification in this trial.

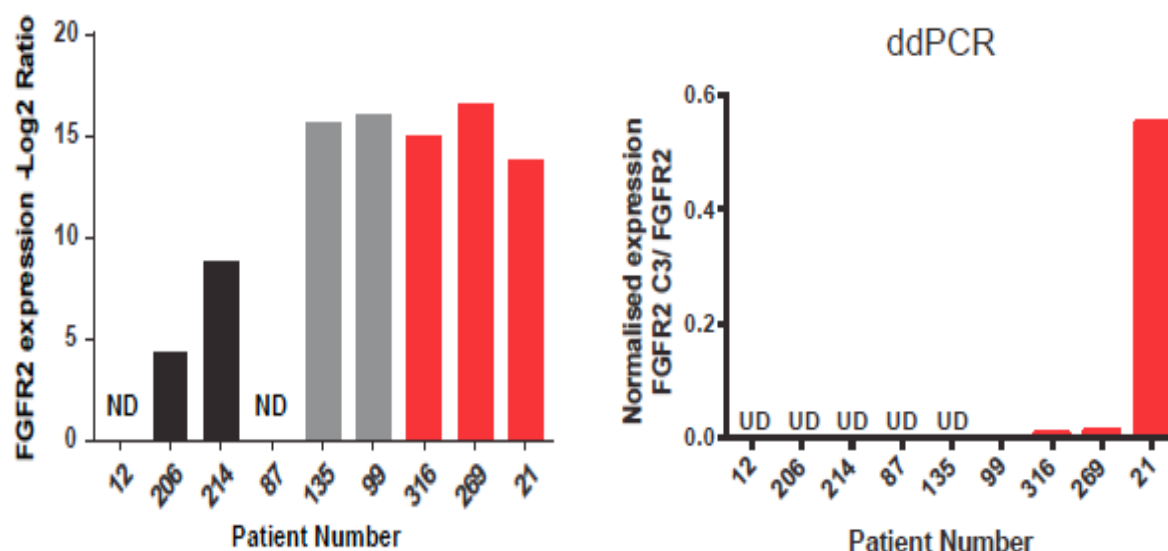




Within FGFR2 amplified cancers, high-level amplification was associated with a substantially higher expression of both FGFR2 mRNA and FGFR2 protein assessed by immunohistochemistry on baseline biopsies.



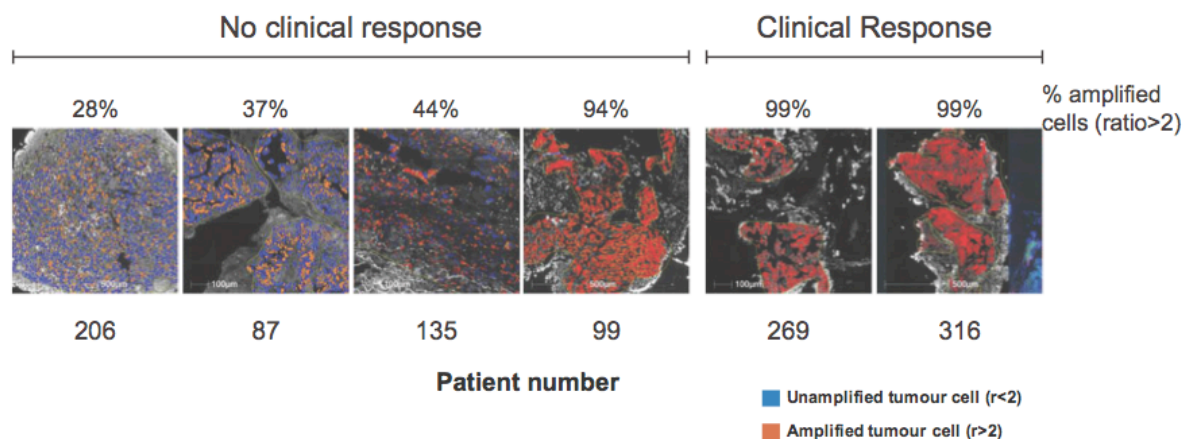
- patients who has a confirmed partial response to AZD4547
- patients with a partial metabolic change on day15 18F-FDG-PET but without a clinical response
- patients without response



Truncated isoforms of FGFR2 have been shown to be potentially important for FGFR2 oncogenic transformation. Only cancers with high level FGFR2 amplification had expression of the truncated FGFR2-C3 isoform although at varying levels suggesting that the C3 isoform was not critical to established cancers. However, expression of FGFR2-C3 may have potential as a biomarker for enrichment of patients likely to respond to FGFR inhibition.

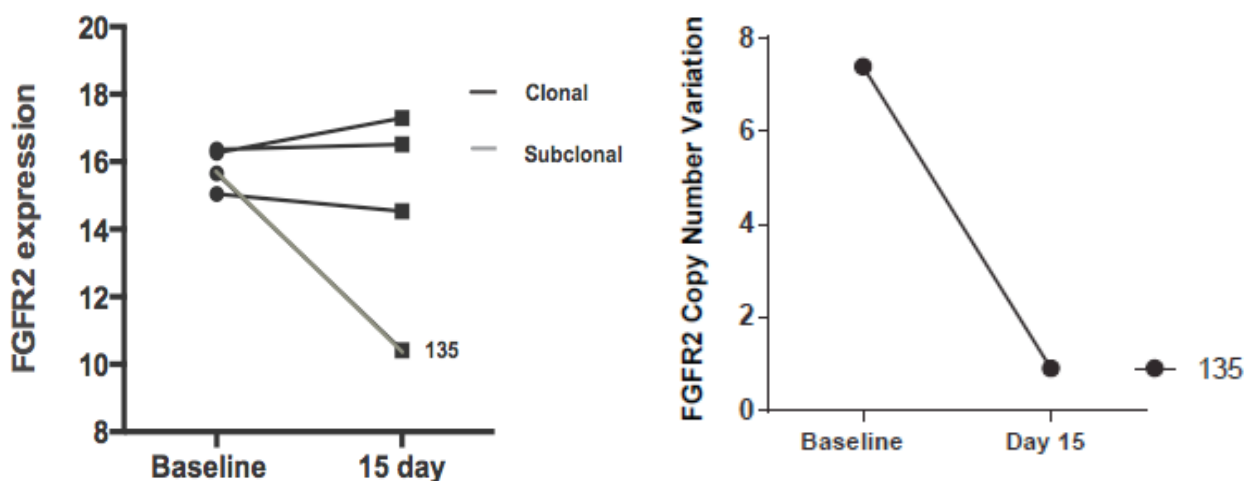
We explored the role of clonality in response to AZD4547 in FGFR2 amplified cancers, using in situ heterogeneity mapping. Two responding patients had clonal homogeneously amplified tumours (>99% tumour cells FGFR2 amplified), the third patient having insufficient residual baseline tissue to make an assessment, whereas all nonresponding patients had tumours with sub-clonal heterogeneous amplification with the presence of non-amplified tumour cells. In particular, heterogeneity in FGFR2 amplification was noted in patient 135 that had a high-level amplified tumour that did not respond to AZD4547. There was limited evidence of heterogeneity and the presence of nonamplified tumour cells in patient 99 that also did not respond. Both patient 135 and 99 had day 15 FDG-PET responses, suggesting that sub-clonality may explain the clinical pattern of FDG response that did not subsequently result in tumour shrinkage and clinical response.





Sections were digitally scanned using the x40 objective of a MIRAX Panoramic 250 Flash II (3D Histech). Tumor was marked and z-stack levels examined for evidence of heterogeneity.

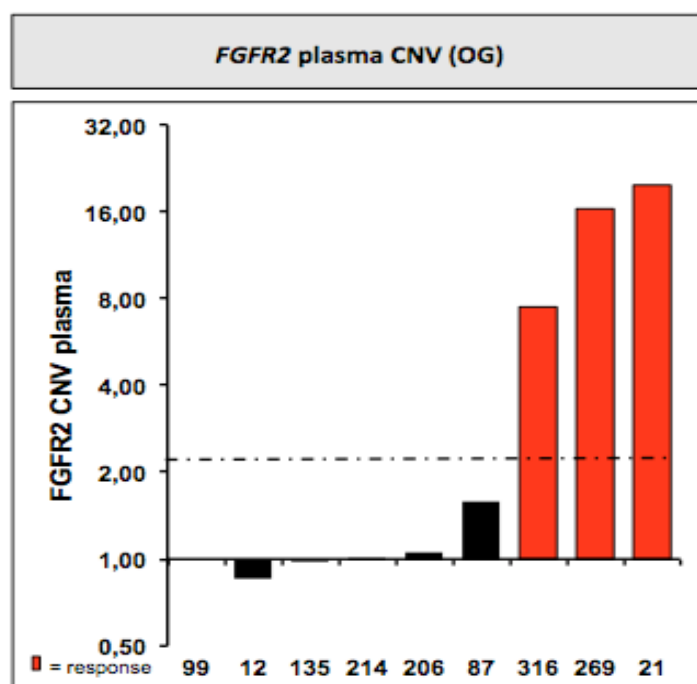
We compared paired gene expression between baseline and day 15 on-treatment biopsies with a custom Nanostring panel. In the heterogeneously amplified tumour from patient 135, high FGFR2 mRNA expression was lost at day 15, whilst in the homogeneously amplified cancers high FGFR2 mRNA expression was maintained at day 15. Similarly, in patient 135 FGFR2 copy number was substantially reduced at day 15.



These findings may reflect the inherent difficulty of sampling in a heterogeneous tumour, but equally could reflect clonal selection by AZD4547. In paired gene expression analysis, the one FGFR1 amplified cancer that responded to AZD4547 had upregulation of estrogen receptor target genes in the day 15 biopsy, suggesting that unregulated estrogen signalling may have limited sensitivity to the FGFR inhibitor in this

tumour.

High-level FGFR2 amplification occurred in only 5% gastric cancers (7/135, arbitrarily defined as FISH ratio>5), a prevalence that may present a barrier to future drug development. We assessed whether screening for amplification in circulating free plasma DNA could identify high-level clonal amplified cancers. FGFR2 copy number was elevated in plasma DNA of all three responding patients, and also in patient 99 who had a response in day 15 FDG-PET. However, FGFR2 copy number was not elevated in plasma DNA of patient 135 with the sub-clonal amplification, and not in low-level amplified cancers, suggesting that plasma assessment has potential to screen for high-level and clonal amplified cancers, to overcome the challenge posed by screening for amplifications on tumour biopsies.



This data suggested that high-level clonal amplification, in particular for FGFR2 amplified cancers, may be required for response to selective FGFR inhibitors, and we investigated why high-level amplification may associate with distinct addiction to FGFR signalling. We showed high-level FGFR2 amplification initiates a distinct oncogene addiction phenotype, characterised by FGFR2 mediated transactivation of alternative receptor kinases, bringing PI3 kinase/mTOR signalling under FGFR control. Signalling in low-level FGFR1 amplified cancers is more restricted to MAPK signalling, limiting sensitivity to FGFR inhibition. Our data provides a mechanistic understanding of the distinct pattern of oncogene addiction seen in highly-amplified cancers and demonstrate the importance of clonality in predicting response to targeted therapy.

**Robust single agent response is only seen in high-level FGFR amplified cancers, with copy number level dictating response to FGFR inhibition both in vitro, in vivo, and in the clinic. High-level amplification of FGFR2 is relatively rare in gastric and breast cancers, and we show that screening for amplification in circulating tumour DNA may present a viable strategy to screen patients.**

After completing the ESMO Fellowship Programme, I have been awarded the Río Hortega Contract (53.732,00€). Grant given by the Instituto de Salud Carlos III, Spain. A two-year contract for training in basic and clinical research under the supervision of Prof. Andrés Cervantes at the Hospital Clínico de Valencia, Spain. I have also been awarded by another grant (3000€) given by the Fundación Mari Paz Jimenez Casado, Madrid (Spain). Project focused on "Activación oncogénica de PI3K/AKT/mTOR y RAS/MAPK en tumores del estroma gastrointestinal (GIST): relevancia biológica e implicaciones terapéuticas" under the supervisión of Dr. César Serrano and Dr. Claudia Valverde at the Vall d'Hebron Hospital, Barcelona, Spain.

**List of Publications Resulting from the Translational Research Project " Phase II multicenter proof of concept study of AZD4547 in FGFR amplified tumours " since October 2013**

The translational results from the FGFR study will be published in *Cancer Discovery* soon. In any case, we presented results as an Oral Abstract Communication at the American Society Of Clinical Oncology Annual Meeting (ASCO) 2015 at Developmental Therapeutics-Clinical Pharmacology and Experimental Therapeutics.

1. Elizabeth Catherine Smyth, Nicholas C. Turner, Clare Peckitt, Alex Pearson, Gina Brown, Sue Chua, Angela Gillbanks, Stephen R. D. Johnston, **Noelia Tarazona**, et al. Phase II multicenter proof of concept study of AZD4547 in *FGFR* amplified tumours (NCT01795768). ASCO 2015, 2508.
2. Pearson A\*, Smyth EC\*, Babina I, Herrera-Abreu MT, **Tarazona N**, et al. High-level clonal FGFR amplification and response to FGFR inhibition in a translational clinical trial. *Cancer Discovery* (Under Revision).

**List of Publications/Presentations from other projects in which I have participated since October 2013**

I combined my activity in the lab with some clinical projects. This has generated a number of publications and conference presentations which are highlighted below:

#### Publications:

1. **Tarazona N**, Smyth EC, Peckitt C, et al. Efficacy and toxicity of salvage paclitaxel chemotherapy in non-Asian patients with advanced oesophagogastric adenocarcinoma. *Ther Adv Med Oncol* 2016; 2: 104-12.
2. Smyth EC, **Tarazona N**, Peckitt C, et al. Exclusion of gastrointestinal cancer patients with prior cancer from clinical trials: Is this justified?. *Clin Colorectal Cancer* 2015; pii: S1533-0028(15)00148-6.
3. Schiavon G, Hrebien S, Garcia-Murillas I, Cutts RJ, Pearson A, **Tarazona N**, et al. *ESR1* mutations evolve during the treatment of metastatic breast cancer and detection in ctDNA predicts sensitivity to subsequent hormone therapy. *Sci Transl Med*. 2015; 7(313):313ra182.
4. Smyth EC, **Tarazona N**, Chau I. Ramucirumab: targeting angiogenesis in the treatment of gastric cancer. *Immunotherapy* 2014;6(11):1177-86.

#### Presentations at International Conferences:

5. Smyth EC, Turner NC, Peckitt C, Pearson A, Brown G, Chua S, Gillbanks A, Johnston SR, **Tarazona N**, et al. Phase II multicenter proof of concept study of AZD4547 in FGFR amplified tumours. 2015 ASCO Annual Meeting, Chicago, EEUU (**Oral Communication**).
6. Schiavon G, Hrebien S, Garcia-Murillas I, Pearson A, **Tarazona N**, et al. *ESR1* mutations evolve during the treatment of metastatic breast cancer and detection in ctDNA predicts sensitivity to subsequent hormone therapy. 2015 AACR Annual Meeting, Philadelphia, EEUU (**Oral Communication**).
7. **Tarazona N**, Smyth EC, Peckitt C, et al. Toxicity and efficacy of salvage paclitaxel chemotherapy in Royal Marsden (RM) oesophagogastric adenocarcinoma (OGA) patients (pts). 2015 GI ASCO Meeting, San Francisco, EEUU (Poster).
8. **Tarazona N**, Smyth EC, Peckitt C, et al. Factors associated with second-line paclitaxel efficacy in non-Asian patients (pts) with advanced oesophagogastric (OG) cancer. 2014 ESMO Congress, Madrid, Spain (Poster).
9. **Tarazona N**, Smyth EC, Peckitt C, et al. Exclusion of patients with prior cancers from clinical trials: Is this justified? 2014 ASCO Annual Meeting, Chicago, Spain (Poster).

#### Award:

★ ESMO 2014 Travel Grant Award

### List of Publications/Presentations resulting from the collaboration with my Home Centre since October 2013

During my fellowship I also continued to be in touch with my home centre in Spain (Hospital Clínico Universitario de Valencia), and I was involved in some projects. From this collaboration has arisen some publications and presentations at International Conferences:

#### Publications:

1. Ibarrola-Villava M, Llorca-Cardenosa M, **Tarazona N**, et al. Deregulation of ARID1A, CDH1, cMET and PIK3CA and target-related microRNA expression in gastric cancer. *Oncotarget* 2015; 6(29):26935-45.
2. **Tarazona N**, Navarro L, Cejalvo JM, et al. Primary paraesophageal Ewing's sarcoma: An Uncommon Case Report and Literature Review. *Onco Targets Ther* 2015; 8:1053-9.
3. Martínez MT, González I, **Tarazona N** et al. Implementation and assessment of a fast-track programme to improve communication between primary and specialized care in patients with suspected cancer: how to shorten time between initial symptoms of cancer, diagnosis and initiation of treatment. *Clin Transl Oncol* 2014; 17(2): 167-72.

#### Presentations at International Conferences:

1. Ibarrola-Villava M, Llorca-Cardenosa MJ, **Tarazona N**, et al. Deregulation of ARID1A, CDH1, cMET and PI3K and target-related microRNA expression in gastric cancer. 2015 ESMO Congress, Vienna, Austria (Poster).
2. Fleitas T\*, Ibarrola-Villava M\*, **Tarazona N**, et al. Altered expression of cMET and PI3CA are associated with primary gastric cancer. 2014 ESMO Congress, Madrid, Spain (Poster).

### Congress attended during the ESMO Fellowship

- \* ESMO 2014: poster presentation  
Topics Gastric Cancer. 651P
- \* 2014 ASCO Annual Meeting: poster presentation  
Category: Health Services Research. Abstract Number: 6544
- \* 2015 Gastrointestinal Cancers Symposium: poster presentation  
Category: Cancers of the Esophagus and Stomach. Abstract Number: 168
- \* ESMO 2015: poster presentation  
Topics Gastric Cancer. 138



### Acknowledgments

I would like to thank the ESMO Fellowship Programme for giving me this great opportunity to continue and reinforce my formation as a translational researcher in Medical Oncology. I would also like to specially thank Prof David Cunningham and Dr Nicholas Turner for taking me in their departments during my Fellowship and for opening my mind to ways to make myself more productive and efficient. During this period, Elizabeth Smyth and Alex Pearson have helped me to improve my clinical and lab skills and have been a big support in this fantastic experience. Before starting the Fellowship I realized the need for more effective therapy in cancer treatment. I had a strong interest in understanding the molecular mechanisms contributing to tumorigenesis. This is the key to discover novel and more efficient therapeutical strategies to tackle this disease. I wanted to be able to exploit basic research in oncology to model disease and target the early events driving cancer development, thus ameliorating the prognosis and reducing the toxicity derived from the chemotherapeutic agents currently used in our everyday practice. These reasons made the ESMO Fellowship Programme the most appropriate to achieve my goals.

Finally, I would like to thank my mentor Prof Andrés Cervantes for his trust in me and for his motivation and encouragement.

This work would not have been possible without the advices and support of them all.



The Breakthrough Breast Cancer Research Centre, Institute of Cancer Research.  
Molecular Oncology Team, June 2015





Prof David Cunningham



Dr Nicholas Turner



Alex Pearson



Elizabeth C Smyth

*“There are no secrets to success. It is the result of preparation, hard work, and learning from failure.” Colin Powell*