ESMO Translational Research Fellowship

(November 2015 – October 2016)

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FINAL REPORT

Host Institute: **Bichat-Beaujon University Hospital, Paris, France**
Mentor: **Professor Sandrine Faivre**
Project title: **Evaluation of MET expression and inhibition in squamous cell carcinoma of the head and neck**

Home Institute: **University Hospital Brno, Brno, Czech Republic**

Introduction

Squamous cell carcinoma of the head and neck (SCCHN) accounts for more than 90% of malignancies arising from the mucosal lining of the oral cavity, pharynx, and larynx. According to 2012 estimates, a total of 686,300 new cases were diagnosed worldwide, and mortality reached up to 55%. The majority of patients still present with advanced disease (stages III and IV), where the prognosis remains dismal. Therefore, novel anticancer therapies guided by reliable biomarkers are urgently needed.

Rationale and Aim

Mesenchymal-epithelial transition factor (c-MET) is a membrane spanning receptor tyrosine kinase primarily found on epithelial cells, while its ligand, hepatocyte growth factor (HGF), is secreted by cells of mesenchymal origin (fibroblasts). The c-MET/HGF signalling pathway promotes cell proliferation, motility, invasiveness, morphogenesis, and angiogenesis, thus having a potential role in cancer development and progression. Typically correlating with poor survival rates, c-MET receptor overexpression has been reported in many tumour types including SCCHN. However, in SCCHN, data on prognostic significance of c-MET have been inconclusive, and trials investigating c-MET inhibition are still scarce.

Primary objectives of our project were to:
(p1) evaluate c-MET immunohistochemical expression in SCCHN patients
(p2) assess whether c-MET receptor may become a new therapeutic target in SCCHN

Secondarily, we aimed to:
(s1) further characterize the c-MET-overexpressing population
(s2) characterize selected SCCHN cell lines with the evaluation of c-MET inhibition
(s3) evaluate c-MET inhibition in fresh tumour explants
(s4) identify new biomarkers for c-MET inhibition

Experimental design

(p1) Evaluation of c-MET immunohistochemical expression in SCCHN patients

(p1a) systematic review of available literature on c-MET immunohistochemistry in SCCHN and meta-analysis of aggregate data

(p1b) retrospective review of patient data in a cohort of 100 cases treated at the Bichat Hospital from 2009 to 2011 including:

- immunohistochemical staining using anti-c-MET antibody at the Bichat Hospital
- manual c-MET quantification at the Bichat Hospital
- quantification of cytoplasmic and membrane receptors separately
- expressing the obtained results as MetMab score or H-score

(p2) Assessment whether c-MET receptor may become a new therapeutic target in SCCHN

(p2a) preparation of an in-depth literature review on c-MET signalling to support the rationale of the project and increase the efficacy of its data collection process (see p1b and s1)

(p2b) review of available literature on c-MET cross-talks with other signalling pathways to analyse possible combinations of c-MET inhibitors with EGFR antagonists

(p2c) research in the field of immunotherapy, emerging as a ground-breaking discovery in oncology, to analyse the results of recent trials with novel drugs which may possibly be exploited in combinations with c-MET inhibitors

(p2d) review of treatment guidelines in recurrent/metastatic SCCHN, as this would probably represent the first cohort of patients to receive a c-MET inhibitor

(p2e) given a growing number of elderly SCCHN patients, further work also focused on issues related to advanced age, since these patients would probably form a substantial part of patients treated with a c-MET inhibitor

(s1) Further characterization of the c-MET-overexpressing population

- this objective makes part of the above mentioned activity (p1b)

(s2) Characterization of selected SCCHN cell lines with the evaluation of c-MET inhibition

- characterisation of protein expression profiles of SCCHN cell lines using Western blot analysis
- identification of cell lines with low and high c-MET expression
- focus on the c-MET signalling pathway including the c-MET receptor and its downstream molecules
- evaluation of both inactive (dephosphorylated) and active forms (phosphorylated) of proteins

(s3) Evaluation of c-MET inhibition in fresh tumour explants

- patients selected from a population undergoing surgical treatment at the Bichat Hospital based on the expected amount of tumour tissue available for testing of c-MET inhibitors (tumours of at least 2 cm in diameter on clinical/radiological examination)
- immediately after surgical excision, the specimens to be transported to the pathology laboratory
- c-MET expression assessed in two phases, before and after exposure to a c-MET inhibitor

(s4) Identification of new biomarkers for c-MET inhibition

- this objective makes part of the above mentioned literature reviews (p1a and p2a)

Results, Conclusions and Future Perspectives

(p1) Evaluation of c-MET immunohistochemical expression in SCCHN patients

(p1a) A systematic review and meta-analysis evaluating c-MET expression on immunohistochemistry in newly diagnosed, non-metastatic SCCHN has been prepared and will be submitted shortly (please see the List of Publication).

(p1b) A data collection of demographic, clinical, and pathological characteristics for the retrospective database of patients operated on SCCHN at the Bichat Hospital has been finished. In order to have a sufficiently long follow-up period for survival analysis, we selected a total of 100 SCCHN patients who underwent surgical interventions, either a curative resection or biopsy, between 2009 and 2011.

In the next step, c-MET immunohistochemical staining on formalin-fixed, paraffin-embedded tissues was carried out. We used the Tissue MicroArray (TMA) technology which was shown to considerably increase the speed of evaluation. Each microarray block consists of approximately 30 separate tissue cores assembled on one histological slide. To minimize subjectivity in our study, the review of TMA slides (3 in total in our study) was performed collectively by two independent pathologists and three oncologists, including me, in one session. Afterwards, the results expressed as MetMab score and H-score will be correlated with the acquired clinicopathological results in our cohort of 100 SCCHN patients.

(p2) Assessment whether c-MET receptor may become a new therapeutic target in SCCHN

(p2a) A comprehensive review focusing on c-MET signalling, both under normal and pathological conditions (e.g. c-MET mutations, copy number alterations, receptor overexpression, inter-pathway cross-talks, and signalling in the context of microenvironmental perturbations), and including also historical data and the results from clinical and laboratory investigations of c-MET inhibitors was prepared and accepted for publication in Critical Reviews in Oncology/Hematology (see the List of Publications).

(p2b) In addition, to investigate potential links between c-MET and EGFR pathways, we prepared and submitted a short article about c-MET-mediated resistance to EGFR inhibitors. Growing evidence suggests that aberrant c-MET signalling may be implicated in primary and secondary resistance to drugs targeting the EGFR receptor. Therefore, we have conducted a literature review to identify clinical studies combining c-MET- and EGFR-directed drugs. The aim was to summarize factors, which are crucial for successful translation of preclinical data to clinical benefit. The paper was accepted by the editorial board and published in the Oral Oncology (see the List of Publications).

(p2c) In the light of recent discoveries in the field of immunotherapy, we analysed the results of the recent phase I-III studies conducted in recurrent/metastatic SCCHN, coming to the conclusion that for immune checkpoint inhibitors progression-free survival does not seem to be a suitable surrogate marker for improved overall survival. This observation was published as a letter to editor in the Oral Oncology (see the List of Publications).

(p2d) If our project was successful and translated into a clinical study, the first cohort of patients to receive a c-MET inhibitor would have recurrent and/or metastatic disease. A review article concerning treatment strategies in patients with recurrent and/or metastatic SCCHN was prepared and published in the Belgian Journal of Medical Oncology. It details various aspects of care in these patients focusing primarily on systemic treatment, which remains the cornerstone of management in most cases (see the List of Publications).

(p2e) Similarly, due to a steadily growing number of SCCHN patients aged over 65 years, a review article was prepared summarizing various aspects of treatment in the elderly, covering all available options from locoregional modalities (surgery, radiotherapy) to systemic approaches (chemotherapy, targeted therapy and immunotherapy). Moreover, special attention was paid to a comprehensive geriatric assessment and geriatric screening tools, which should be integrated in clinical trials enrolling senior patients. The article was published in the Frontiers in Oncology (see the List of Publications).

(s1) Further characterization of the c-MET-overexpressing population

In the cohort of 100 SCCHN cases retrospectively retrieved from the database of the Bichat Hospital, the age at diagnosis ranged between 31 and 88 years. SCCHN of the oral cavity, oropharynx, hypopharynx, and larynx were included together with one case of maxillary sinus cancer. Both primary and recurrent tumours were evaluated. No restrictions were placed on gender, TNM stage, or the grade of differentiation. As tobacco abuse and alcohol consumption are well recognised risk factors for SCCHN, these have been added to the clinical patient characteristics. Moreover, to expand the information about tumour specimens, the following histopathological features were acquired: surgical margins (negative or microscopically positive), vascular embolism, perineural invasion, and extracapsular spread. The results are planned to be further analysed.

(s2) Characterization of selected SCCHN cell lines with the evaluation of c-MET inhibition

Initially, we studied four cell lines (Hep2, SQ20B, SCC61, and Detroit 562). Following protein extraction we were able to precisely characterize the expression profiles using Western blot analysis. To study the molecular cascade of c-MET in both active and inactive forms, the first group of samples was derived from cell lines treated with hepatocyte growth factor (HGF), which represented the only confirmed ligand for the c-MET receptor, while the second one served as a control. The protocol included the following proteins: epidermal growth factor receptor (EGFR, 170 kDa), c-MET (β-subunit of 145 kDa), E-cadherin (135 kDa), GRB2-associated-binding protein 1 (GAB1, 110 kDa), vimentin (57 kDa) as well as their phosphorylated counterparts (p-MET and p-Gab1) and two additional downstream molecules (p-AKT and p-ERK). In both groups, actin was used as a loading control for Western blotting.

Afterwards, we selected one cell line with low c-MET expression and another one with high c-MET expression. Samples from both cell lines were exposed to HGF and two different c-MET inhibitors. The results are planned to be presented together with the results of c-MET inhibition in fresh tumour explants (objective s3).

(s3) Evaluation of c-MET inhibition in fresh tumour explants

Tumour samples of selected patients were transported to the laboratory, where they were exposed for 48 hours to the following anticancer drugs:

1) A highly specific c-MET tyrosine kinase inhibitor 1 microM
2) A highly specific c-MET tyrosine kinase inhibitor 5 microM
3) Cisplatin 1 microM
4) Cetuximab
+ control samples (no exposure)

At present, 3 cases were received safely in the laboratory and fulfilled all quality requirements. The process will continue to reach a target number of about 10-15 cases. The results will be presented together with objective (s2).

(s4) Identification of new biomarkers for c-MET inhibition

As a result of the above mentioned data analysis (p1a and p2a) we came to the conclusion that immunohistochemical c-MET expression represents a promising biomarker to guide targeted therapy with c-MET inhibitors.

Final conclusions and future perspectives

Based on the results of our extensive literature review, clinical and laboratory work, it follows that the c-MET receptor may indeed become a promising druggable target in SCCHN, either using single-agent specific inhibitors or combination therapies. These findings have a particular importance within the context of future clinical research, as further studies are needed to prospectively verify the relevance of c-MET in biomarker-defined patient populations.

List of Publications and Presentations Resulting from the Translational Research Project "Evaluation of MET expression and inhibition in squamous cell carcinoma of the head and neck"

PUBLICATIONS

1. Szturz P, Raymond E, Abitbol C, Albert S, de Gramont A, Faivre S. Understanding c-MET signalling in squamous cell carcinoma of the head and neck. Critical Reviews in Oncology and Hematology. doi: 10.1016/j.critrevonc.2017.01.004
(Impact Factor = 5.039)

2. Szturz P, Raymond E, Faivre S. c-MET-mediated resistance to EGFR inhibitors in head and neck cancer: How to move from bench to bedside. Oral Oncol. 2016; 59: e12-4. doi: 10.1016/j.oraloncology.2016.05.017.
(Impact Factor = 4.286)

3. Szturz P, Faivre S. Letter to the editor referring to the publication entitled "The role of antagonists of the PD-1:PD-L1/PD-L2 axis in head and neck cancer treatment" by Pai et al. Oral Oncol. 2016; 62: e3-e4. doi: 10.1016/j.oraloncology.2016.08.007.
(Impact Factor = 4.286)

4. Szturz P, Vermorken JB. Recurrent and metastatic non-nasopharyngeal head and neck cancer: state of the art of systemic treatment. Belg J Med Oncol. 2016; 10: 207-214.

5. Szturz P, Vermorken JB. Treatment of Elderly Patients with Squamous Cell Carcinoma of the Head and Neck. Front Oncol. 2016; 6: 199. doi: 10.3389/fonc.2016.00199.

6. Szturz P, Budíková M, Vermorken JB, Horová I, Gál B, Raymond E, de Gramont A, Faivre S. Prognostic value of c-MET and its association with various clinicopathological parameters in head and neck cancer: a systematic review and meta-analysis of aggregate data. In preparation.

PRESENTATIONS

7. Szturz P. Évaluation de l'expression de MET et son inhibition dans les carcinomes épidermoïdes des voies aéro-digestives supérieures (VADS). Research group meeting at the Hôpital Lariboisière, Paris, 18. 11. 2015.

8. Szturz P. Évaluation de l'expression de MET et son inhibition dans les carcinomes épidermoïdes des voies aéro-digestives supérieures (VADS). Interdisciplinary staff meeting at the Hôpital Beaujon, Paris, 15. 2. 2016.

9. Szturz P. Récapitulation du METNECK projet. Research group meeting at the Hôpital Lariboisière, Paris, 19. 10. 2015.

List of Publications and Presentations resulting from other projects during the fellowship period (if applicable)

1. Szturz P, Specenier P, Van Laer C, Van Den Weyngaert D, Corthouts B, Carp L, Van Marck E, Vanderveken O, Vermorken JB. Long-term remission of locally recurrent oropharyngeal cancer after docetaxel-based chemotherapy plus cetuximab. Eur Arch Otorhinolaryngol. 2016; 273: 1629-1636.
(Impakt Factor = 1.627)

2. Vanderveken OM, Szturz P, Specenier P, Merlano MC, Benasso M, Van Gestel D, Wouters K, Van Laer C, Van den Weyngaert D, Peeters M, Vermorken J. Gemcitabine-Based Chemoradiation in the Treatment of Locally Advanced Head and Neck Cancer: Systematic Review of Literature and Meta-Analysis. Oncologist. 2016; 21: 59-71.
(Impakt Factor = 4.789)

3. Szturz P, Vermorken JB. Systemic Treatment of Recurrent/Metastatic Squamous Cell Carcinoma of the Head and Neck. In: Bernier J, editor. Head and Neck Cancer: Multimodality Management (2nd edition). Cham: Springer International Publishing AG 2016: 711-729.

4. Szturz P, Vermorken JB. Immunotherapeutic Approaches. In: Vermorken JB, Budach V, Leemans CR, Machiels JP, Nicolai P, O'Sullivan B (eds.). Critical Issues in Head and Neck Oncology: Key concepts from the Fifth THNO Meeting. Cham: Springer International Publishing AG 2017: 233-249.

5. Szturz P, Vermorken JB. Treatment in the Elderly. In: Vermorken JB, Budach V, Leemans CR, Machiels JP, Nicolai P, O'Sullivan B (eds.). Critical Issues in Head and Neck Oncology: Key concepts from the Fifth THNO Meeting. Cham: Springer International Publishing AG 2017: 251-261.

6. Adam Z, Petrášová H, Řehák Z, Koukalová R, Krejčí M, Pour L, Vetešníková E, Čermák A, Ševčíková S, Szturz P, Král Z, Mayer J. [Evaluation of five years of treatment of Erdheim-Chester disease with anakinra: case report and overview of literature]. [Article in Czech]. Vnitr Lek. 2016; 62: 820-832.

7. Adam Z, Šedivá A, Koukalová R, Řehák Z, Petrášová H, Szturz P, Adamová Z, Vetešníková E, Pour L, Krejčí M, Sandecká V, Pourová E, Čermáková Z, Ševčíková S, Král Z, Mayer J. [Schnitzlers SyndromeDifferential diagnostics, an overview of therapeutic options and description of 5 cases treated with anakinra]. [Article in Czech]. Vnitř Lék. 2016; 62: 713-727.

8. Adam Z, Szturz P, Krejčí M, Koukalová R, Michalková E, Řehák Z, Pourová E, Pour L, Volfová P, Sandecká V, Čermáková Z, Křen L, Sokol F, Hanke I, Penka I, Petrášová H, Ševčíková S, Král Z, Mayer J. [Treatment of 14 cases of Castlemans disease: the experience of one centre and an overview of literature]. [Article in Czech]. Vnitř Lék. 2016; 62: 287-298.

9. Koukalová R, Szturz P, Svobodová I, Stulík J, Řehák Z. [Localized Amyloidosis Involving the Nasal Cavity]. [Article in Czech]. Klin Onkol. 2016; 29: 216-219.

Selection of Courses and Workshops Attended During the Fellowship

1. Fifth Trends in Head and Neck Oncology (THNO), Lisboa, Portugal, 5-7. 11. 2015.

2. Workshop “Introduction à la qualité, à la norme ISO 9001 et à l’analyse de risque”. AFR Oncology, Paris, 6. 4. 2016.

3. Workshop “Analyse de risque: Concepts et méthodes”. AFR Oncology, Paris, 4. 5. 2016.

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