ESMO Translational Research Fellowship

(November 2015 – November 2017)

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FINAL REPORT

Host Institute: **Institute Gustave Roussy, Villejuif, France**

Mentor: **Dr. Bernard Escudier**

Project title: **The BRAVE Projet**
Brain metastases and panc**R**eatic metastases: A clinic-pathological comparison of **V**arious facets of the tumor h**E**terogeneity in renal cell carcinoma (RCC).

Introduction

As expression of tumour heterogeneity in RCC, brain (BM) and pancreatic metastases (PM) look like the two faces of the same coin: they arise from the same tumour type but they have reverse prognosis. BM have been reported in 16% of RCC with unfavorable prognosis (median overall survival: 13 months) but PM have been reported in 10% of RCC and have been associated with long term survival (median overall survival: 39 months) (Keira 2015; Grassi 2015). Today, no identifiable parameters can reliably predict the risk of BM or PM formation but evidence from other tumour types shows that distinct molecular tumour phenotypes may be associated with an increased risk of brain metastasis. Therefore, we hypothesized that clinical and pathological factors or biomarkers expression may vary significantly throughout the metastases sites (BM and PM), in the primary tumour versus metastases (BM or PM) and finally throughout the primary tumours, and potentially force the predictive value of one of these factors. Farther, this knowledge will be important to determine whether the development of optimal predictive models can be conducted on tissue from metastatic sites or whether primary tumour tissue is likely to be more informative. For these reasons, we performed an extensive analysis of biomarkers expression in a series of metastasis to brain and pancreas after surgical resections and when it was available in the corresponding primary tumour too.

Experimental design

All patients with radiological diagnosis of BM and/or PM from RCC have been assessed for eligibility from the RCC French database (from 5 French institutions)
Tissue specimens of histologically confirmed RCC were retrieved by between November 2015 and October 2016. Only RCC patients who underwent nephrectomy, pancreatectomy or neurosurgical resection and with available histological tissue samples were enrolled. Patient characteristics, treatments and outcome data were collected.
To understand the occurrence of BM or PM from RCC patients, we proposed 3 steps:
i) Correlation between clinico-pathological characteristics and metastasis location
Patients characteristics, treatments and outcome data were retrospectively collected.

Characteristics were described at diagnosis and by groups (patients with a diagnosis of BM were defined in BRAIN group, patients with a diagnosis of PM were defined in PANCREAS group; when a patient had both, the group was defined by the site of metastasis that occurs firstly)

ii) Histopathologic features description by revision of samples

Two urologic pathologists centrally reviewed all available microscopic slides according to the 1998 UK Royal College of Pathologist Guidelines, in a blinded fashion. Primary and metastatic tumours from the same patient were reviewed separately. Primary tumors characteristics were compared between patients with BM vs PM. The pathologic features studied included histologic subtype, eosinophilic cytoplasm, tumour necrosis, sarcomatoid differentiation, Vascular Invasion (VI), lymphocytic infiltrate (TIL) and presence of haemorrhage. For primary tumours, Fuhrman grade was also described.

iii) IHC and FISH analyses in BRAIN, PANCREAS and primary nephrectomy samples

PD-1, PD-L1, ALK and c-MET protein expression was determined by IHC. IHC for PD-L1 (monoclonal mouse antibody, E1L3N) was performed using Cell Signaling Technology.

IHC for MET (monoclonal mouse antibody, SP44) was performed using a Ventana staining system.

PD-L1 expression

Immunostaining present (> 1%) or absent in tumor cells (TC) and immune cells (IC).

MET expression

Positive if ≥ 50% of TC showed a moderately dense staining.

Evaluation of IHC

The positivity of markers was described:

- inter-patients, considering all available primary tumors and metastases;
- intra-patients, analyzing paired samples.

ALK and MET gene status and copy number alteration of chromosome 7 were studied with FISH.

Statistical analyses

For the three steps, comparison of demographic and patho-biologic features between BM and PM were evaluated using Chi2 or Fisher's exact test for categorical data and Kruskal-Wallis test for continuous data. Overall survival (OS) was estimated by Kaplan-Meier method. Statistical analyses were carried out using SAS version 9.4.

Results, Conclusion and Future Perspectives

Between November 2015 and September 2016, 380 patients with radiological diagnosis of BRAIN and/or PANCREAS have been assessed for eligibility from the RCC French database. Only RCC patients who underwent surgical resection/biopsy of BRAIN or PANCREAS and with available (metastases and/or primary) histological tissue samples were enrolled. Today, not all samples are available and not all samples have been analysed.

i) Correlation between clinic-pathological characteristics and outcome:

Population

- 184 patients were included in this study. The median age was 54 years with a majority of men (70%). Most tumors (99%) were clear-cell histology.
- 47% had a diagnosis of first metastasis to any other location than brain/pancreas (26% to lung; 22% to any other location).
- 27% underwent at least one metastasectomy before brain/pancreas surgery.

Characteristics by metastasis location

When shared by groups, 101 (55%) patients were BRAIN and 83 (45%) PANCREAS. As expected based on literature reports, the proportion of BRAIN was higher than PANCREAS group.
Between BRAIN and PANCREAS patients, there were no significant differences in baseline characteristics (sex, age at diagnosis of RCC, nephrectomy, primary RCC (left vs right), histology, Fuhrman grade, metastasectomy) except for T stage. Forty-three (58%) patients in the BRAIN group had a T3-T4 stage vs 22 (30%) patients in the PANCREAS group (p= 0.0012) (Table 1).

Table 1. Characteristics at diagnosis

		BRAIN, n (%) 101 (55%)	PANCREAS, n (%) 83 (45%)	p value#	All, n (%) 184 (100%)
Year of diagnosis*					
	1981-2000	21 (21%)	48 (58%)	**<.0001**	69 (37%)
	2001-2006	39 (39%)	22 (26%)		61 (34%)
	2007-2016	41 (41%)	13 (16%)		54 (29%)
Gender	**Male**	73 (72%)	55 (66%)	0.3778	128 (70%)
	Female	28 (28%)	28 (34%)		56 (30%)
Age	**At diagnosis**				
	median [range]	53.4 [27.8-79.7]	54.8 [34.2-79.4]	0.3366	54 [27-79]
Site of Primitive Tumour					
	Right	46 (49%)	45 (55%)	0.7083	91 (52%)
	Left	46 (49%)	35 (43%)		81 (46%)
	Bilateral	2 (2%)	2 (2%)		4 (2%)
	NA*	7*	1*		8 *
Nephrectomy					
	No	8 (8%)	0 (0%)	**0.0075**	8 (4.5%)
	Partial/Total	88 (92%)	82 (100%)		170 (95.5%)
	NA*	5*	1*		6 *
Tumor Histology					
	Clear cell	99 (99%)	82 (99%)	0.8945	182 (99%)
	Papillary	1 (1%)	1 (1%)		2 (1%)
Tumour (TNM)					
	T1-T2	31 (42%)	51 (70%)	**0.0012**	82 (56%)
	T3-T4	43 (58%)	22 (30%)		65 (44%)
	NA*	27*	10*		37 *
Fuhrman					
	I-II	23 (33%)	31 (50%)	**0.0456**	54 (41%)
	III-IV	47 (67%)	31 (50%)		78 (59%)
	NA*	31*	21*		52*

Comparing patients characteristics at time to BRAIN or PANCREAS formation (Table 2), there were no differences in presence of metastases or metastasectomy before brain/pancreas metastasis, but BRAIN patients have frequently a diagnosis of lung metastasis before brain/pancreas metastasis (38% BRAIN vs 11% PANCREAS (p= 0.0002). Interestingly, time to BRAIN was shorter than time to PANCREAS (median: 3.66 vs 8.44 years, p<.0001).

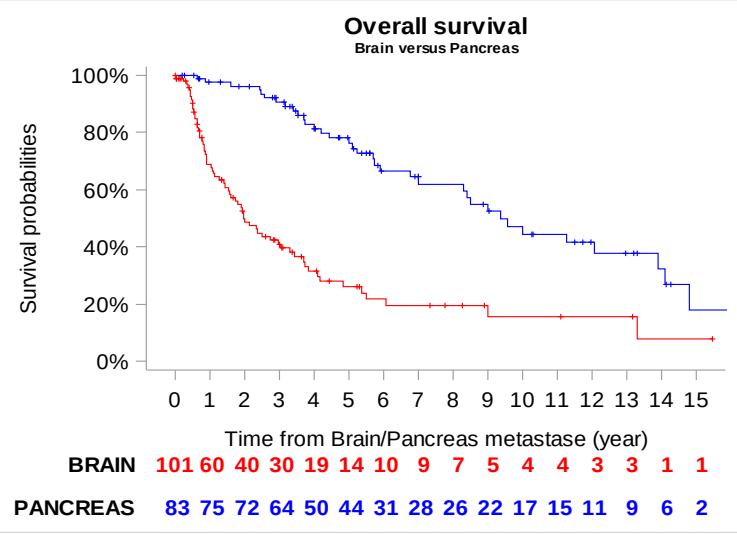
Table 2. Characteristics at Brain or Pancreas metastasis

	BRAIN, n (%) 101 (55%)	PANCREAS, n (%) 83 (45%)	p value#	All, n (%) 184 (100%)
Metastases before Brain/Pancreas metastasis				
No	45 (44%)	52 (44%)	0.076	97 (53%)
Yes	56 (56%)	31 (38%)		87 (47%)
Lung	38 (38%)	9 (11%)	0.0002§	47 (26%)
Other	18 (18%)	22 (27%)		40 (22%)
Metastasectomy before				
No	75 (74%)	60 (72%)	0.7638	135 (73%)
Yes	26 (26%)	23 (28%)		49 (27%)
Time to Brain/Pancreas metastasis, median [range]				
	3.66 [0-15.53]	8.44 [0-22.72]	**<.0001**	5.8 [0-22]
Time to First metastasis, median [range]				
Brain/Pancreas	2.15 [0-15.53]	8.41 [0-22.72]	**<.0001**	5.5 [0-22]
Others	1.99 [0-15.01]	4.51 [0-11.01]		2.8 [0-15]

Survival analysis

▪ In the overall population set of 184 patients, the number of deaths was 101 (55%). Patients with BRAIN have a shorter OS [median: 1.97 months 95% CI (1.44–3.03) versus 9.35 (7.01–13.88, P < 0.0001] than patients with PANCREAS. (Figure 1.)

Figure 1. Survival by groups



ii) Histopathologic features description by revision of samples (BM, PM and primitive tumour)

RCC samples were recovered from different institutions (Mareseille, Lyon, George Pompidou, Foch and Caen Hospitals et others small centres in France).

181 resected RCC specimen were successful collected (42 primary tumours and 138 metastases). Clinicopathologic characteristics were assessed by revision of samples. Factor considered were:

histopathology (clear cell vs non clear cell), Fuhrman grading classification (from I to IV), presence of a sarcomatoids component (Yes/No), presence of necrosis (Yes/No), the microscopic vascular invasion Yes/No), Tumor-infiltrating lymphocytes (TILs) (absent/low, med/high), and bleeding (Yes/No).

Histopathologic features description

Most of primary tumor specimens reviewed were clear cell histology (100%), without eosinophilic cell morphology (52%), grade 2 of Fuhrman (63%), without necrosis (52%), moderate Tumor Inifiltrate Lymphocytes (TIL) (49%) and hemorrhage (65%). Throughout the primary tumours, we observed a difference in the presence of TIL: moderate/high in 84% of primary tumors of patients who developed BM and low in 44% of primary tumors of patients who developed PM (p< 0.001) (Table 3).

Table 3. Histology review of 43 primary tumors and repartition by brain vs pancreatic metastasis development

		Primary RCC, n (%)	BM, n (%)	PM, n (%)	p value
Histology					
	Clear cell	43 (100)	25 (58)	18 (42)	
	Eosinophilic Cell Morpho.				
	No	22 (52)	12 (48)	10 (56)	0.267
	Yes	21 (48)	13 (52)	8 (44)	
	Papillary	-	-	-	
Fuhrman					
	I	-	-	-	
	II	11 (25)	6 (24)	5 (27)	
	III	27 (63)	15 (60)	12 (67)	**0.080**
	IV	5 (12)	4 (16)	1 (6)	
Necrosis					
	No	22 (52)	11 (44)	11 (61)	**0.020**
	Yes	21 (48)	14 (56)	7 (39)	
Sarcomatoid features					
	No	41 (95)	23 (92)	18 (100%)	**0.004**
	Yes	2 (5)	2 (8)	-	
Vascular Invasion					
	No	28 (65)	14 (56)	14 (78)	**0.001**
	Yes	15 (36)	11 (44)	4 (22)	
Tumor infiltrates lymphocytes					
	Low	12 (28)	4 (16)	8 (44)	**0.001**
	Moderate	21 (49)	12 (48)	9 (50)	
	High	10 (23)	9 (36)	1 (6)	
Hemorrhage					
	No	15 (35)	8 (32)	7 (39)	0.309
	Yes	28 (65)	17 (68)	11 (61)	

Between BM and PM, we observed statistically significant discordance of eosinophilic cell morphology, necrosis, sarcomatoid features, TIL and grade of hemorrhage (Table 4).

Table 4. Histology review of 87 brain vs 51 pancreatic metastases

		BM, n (%)	PM, n (%)	p value
Histology				
	Clear cell	85 (98)	51 (100)	0.155
	Eosinophilic Cell Morpho.			
	No	49 (57)	41 (80)	**0.001**
	Yes	36 (43)	10 (20)	
	Papillary	2 (2)	-	
Necrosis				
	No	18 (21)	41 (80)	**0.001**
	Yes	69 (79)	10 (20)	
Sarcomatoid features				
	No	82 (94)	51 (100)	**0.013**
	Yes	5 (6)	-	
Vascular Invasion				
	No	87 (100)	49 (96)	0.151
	Yes	-	2 (2)	
Tumor infiltrates lymphocytes				
	Low	27 (31)	26 (51)	**0.006**
	Moderate	43 (50)	21 (41)	
	High	17 (19)	4 (8)	
Hemorrhage				
	No	16 (18)	24 (47)	**0.001**
	Yes	71 (82)	31 (53)	

iii) IHC and FISH analyses in BRAIN, PANCREAS and primary nephrectomy samples

Inter-patients IHC

Considering all specimens, PD-L1 TC and IC were expressed in 22% and 51% of cases, respectively. MET was positive in 23% of cases. We observed a lower expression of PD-L1 TC and MET in primary RCC compare to metastases and a significant higher expression of MET in BM compared to PM (Table 5).

Intra-patients IHC

35 specimens were paired samples from same patients (primary-metastasis); 20 were BM and 15 were PM respectively paired with their primary.

Table 5. Inter-patients biomarker expression

Biomarker expression	Primary RCC	Metastases				All specimens
		PM	BM	p value	All	
All sample (n=183)						
PD-L1 TC	12%	19%	**23%**	0.63	21%	22%
PD-L1 IC	51%	49%	**47%**	0.82	47%	51%
MET	0%	2%	**35%**	<0.001	24%	23%

Comparing paired samples (primary-metastasis), there was discordance of PD-L1 in TC or IC and of MET expression in 30%, 27% and 24% of samples, respectively. PD-L1 discordance was higher in the 80% of cases were BM or PM were metachronous (> 6months). The discordance of PD-L1 TC, IC and MET between primary tumor and PM/BM was 15%/40%, 33%/22% and 0%/67%, respectively (Table 6).

Correlation of IHC findings with histopathologic features

PD-L1 positivity (TC and IC) was correlated to higher Fuhrman grade (p<0.02), PDL-L1 IC to TIL (p<0.02) and MET to TIL (p=0.013) and to necrosis (p<0.001).

Table 6. Intra-patients biomarker expression

Biomarker expression	Discordance between primary tumor and metasteses		
	PM	BM	p value
Paired samples (n=35)			
PD-L1 TC	15%	**40%**	
PD-L1 IC	33%	**22%**	<0.001
MET	0%	**67%**	

In conclusion

This project describes for the first time tumour heterogeneity in RCC by clinical, pathological and biological aspects, focusing on differences between brain and pancreatic metastases. Previous study have shown that PD-L1 and c-MET, are not predictive biomarkers, but only prognostic factors. In this large analysis, we found that RCC is characterized by an inter and intra-tumor heterogeneity in terms of histopathological features, and of PD-L1 and MET expression between primary and metastases (brain/pancreatic lesions). Our data support to develop clinical studies that will investigate the predictive biomarkers analyzing primary and metastatic lesions.

Outlook

FISH analysis and PD-1 and ALK IHC are still on-going.

List of Publications and Presentations Resulting from the Translational Research Project "Brain metastases (BM) and pancReatic metastases (PM): A clinic-pathological comparison of Various facets of the tumor hEterogeneity in renal cell carcinoma (RCC)"

- **Oral Presenter at** *Immunoncology summit 2017* in Paris**.** http://www.crc.jussieu.fr/immun_oncology_summit_2017_myltenlyi_agenda.pdf
- **Oral Presenter and Merit Award recipient** at The *5 th Pavia International symposium on advanced kidney cancer* in Pavia (November 2016).
- **Poster presenter** at ASCO 2017 https://meetinglibrary.asco.org/record/144768/abstract
- **Poster presenter** at ASCO 2016 https://meetinglibrary.asco.org/record/122755/abstract

- **Poster presenter** at ESMO 2016 https://oncologypro.esmo.org/Meeting-Resources/ESMO-2016/Brain-and-pancreatic-metastases-A-clinico-pathological-comparison-of-various-facets-of-tumor-heterogeneity-in-renal-cell-carcinoma-The-BRAVE-project
- **Oral Presenter** at *The 5th World Top Communications of the Year in Genito-Urinary Oncology* at Arezzo (November 2016). http://events.startpromotion.it/site/common/uploads/Programma_Def_5th_WTCGU_Arezzo_2016.pdf
- **Oral presenter** at *Supernovae in oncologia* in Pisa (November 2016).
- **Oral Presenter** at *Il tempo dell'immunoterapia nel trattamento dei tumori renali: tra stato dell'arte e prospettive della pratica clinica* in Arezzo (September 2016).
- **Oral presenter** at *The SPECIAL CARE* in Rome (February 2016).

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

- **Oral Presenter** at *The 13th European International Kidney Cancer Symposium* in Prague Czech Republic (April 2018) http://euikcs.com/kca/prague2018/docs/agenda.pdf
- **Poster presenter and Merit Award recipient** at ASCO 2018 https://meetinglibrary.asco.org/record/160570/abstract
- **Poster presenter and Merit Award recipient** at ASCO Genitourinary cancer Symposium 2017 https://meetinglibrary.asco.org/record/140186/abstract
- **Poster presenter** at ASCO Genitourinary cancer Symposium 2017 https://meetinglibrary.asco.org/record/140317/abstract
- **Poster presenter and Merit Award recipient** at ASCO 2017 http://ascopubs.org/doi/abs/10.1200/JCO.2017.35.15_suppl.3015
- **Oral presenter** at ESMO 2017 https://oncologypro.esmo.org/Meeting-Resources/ESMO-2017-Congress/Renal-cancer-Sequence-of-therapies-immunotherapy-case-presentation
- **Poster presenter** at ESMO 2017 https://oncologypro.esmo.org/Meeting-Resources/ESMO-2017-Congress/Efficacy-of-cabozantinib-C-after-PD-1-PD-L1-checkpoint-inhibitors-in-metastatic-Renal-Cell-Carcinoma-mRCC-the-Gustave-Roussy-experience
- **Oral presenter** at Lung Cancer a new vision symposium in Lyon (April 2017). file:///C:/Users/L_DEROSA/Downloads/BMS_18833_SYMPOSIUM_LUNG_CANCER_PROGRAMME_APRIL_2017_V9_EXE_ENG_Preview.pdf
- **Oral presenter** at GONO, 29° anniversario della Fondazione. Tra clinica e ricerca in oncologia: come il laboratorio ed i risultati della ricerca entrano nei nostri ambulatory (November 2016) http://gonogroup.org/wp-content/uploads/2016/05/Congresso-2016.pdf
- **Derosa L,** at al. Oncoimmunology 2018 https://www.ncbi.nlm.nih.gov/pubmed/29872574
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Acknowledgements

Before my fellowship, I knew I wanted to conduct research in cancer and get involved in oncology in ways other than direct clinical care. But, I didn't know how or where to begin. The fellowship opened my eyes to the breadth of medical oncology. I was exposed to multiple examples of collaborations across disciplines for the purposes of research and teaching, including, for example, clinical and translational trials development, clinical research and basic science. The fellowship provided me with the opportunity to "try out" all these different roles. From my mentor, Dr. Bernard Escudier, I received exceptional, personalized mentoring in hypothesis generation and in all the steps of manuscript writing and oral presentation. This experience provided me with the confidence to publish my own work and present it at international scientific meetings. Without question the fellowship was instrumental to launching my career as an academic oncologist.

What I most appreciate about my experience in the fellowship was being surrounded by a group of colleagues who were passionate and truly generous with their knowledge, ideas, and time. Their goal was to see us go out into the broader world—whether into practice or academia—and thrive. I didn't know what to expect when I first arrived but I can't say enough about how wonderful they are. They made for an incredibly stimulating place to live and work.

At the GU Group dinner in Paris with my colleagues

This ESMO Translational Fellowship Research Project was supported by an educational grant from