Sunitinib impact on Kinome profiles of Peripheral Blood Mononuclear Cells from renal cell carcinoma patients: do molecular effects correlate with clinical data?

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Abstract #2038

BACKGROUND

Sunitinib, a potent multi-tyrosine kinase (TK) inhibitor, is a standard first-line treatment for metastatic renal cell carcinoma (mRCC). Sunitinib is known to have immune-modulating properties especially on regulatory T-cells and tumor-infiltrating lymphocytes. However, data is sparse about sunitinib impact on peripheral lymphocytes.

OBJECTIVES

To investigate the *ex vivo* effect of sunitinib and its active metabolite SU12662 on peripheral blood mononuclear cells (PBMC) from naïve mRCC patients using a high throughput kinomic profiling method.

METHODS

1- Retrospective study

- 88 mRCC patients under sunitinib therapy from June 2006 to January 2015
- Baseline clinical and biological parameters were collected
- Haematological specifications were gathered at Day 0 and Day 21 (expressed as a ratio D21/D0) of the first cycle of treatment
- Progression Free Survival (PFS) was estimated with the Kaplan-Meïer method
- Factors with a p-value lower than 0.10 in univariate cox model were entered into a multivariate analysis.

2- Kinomic study

- 21 naïve mRCC patients and 12 healthy volunteers
- TK activity profiles of PBMC lysates were generated on TK PamChip® microarrays (Figure 1)
- The *ex vivo* effect of sunitinib and its active metabolite SU12662 were determined in PBMC from mRCC patients.
- All data were analyzed using BioNavigator ® software.

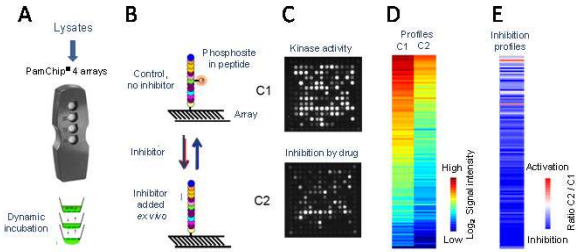

Figure 1 : Kinase activity profiling of PBMC lysates on TK PamChip® microarrays (A). Phosphorylation of peptides is monitored (B, C) in absence or in presence of *ex vivo* inhibitor (C1 and C2 conditions). Signal intensities are converted to kinase activity profiles (D) and to inhibition profiles (E) by calculation of ratio of C2 and C1.

RESULTS

1- Association between increased D21/D0 total peripheral lymphocytes ratio and shorter PFS

- Median PFS : 234 days (confidence interval, CI95%, 179-289).
- Lymphocyte D21/D0 ratio; ECOG 0-1 and Body Mass Index (BMI) were independently associated with PFS (Table 1).

Table 1 : Results of univariate and multivariate analysis of PFS

Variables (units)	Univariate analysis		Multivariate analysis	
	Hazard Ratio [CI95%]	p-value	Hazard Ratio [CI95%]	p-value
Male Sex		Ns		
Age (years)		Ns		
ECOG 0-1	0.56 [0.31-1.01]	**0.053**	0.44 [0.23-0.84]	**0.0135**
Lean body mass (kg)	0.96 [0.94-0.98]	**0.0003**		Ns
BMI (kg/m²)	0.87 [0.82-0.93]	**<0.0001**	0.86 [0.80-0.93]	**0.0001**
Metachronous metastasis		Ns		
RCC Histological type	0.44 [0.26-0.75]	**0.0025**		Ns
Fuhrman's grade 4	2.20 [1.15-4.19]	**0.0167**		Ns
Nephrectomy	0.31 [0.15-0.62]	**0.0010**		Ns
Heng score Favourable (n=6) Intermediate (n=50) Poor (n=32)	 0.33 [0.12-0.97] 0.54 [0.33-0.89] 1	**0.0216**		Ns
Increased LDH		Ns		
Steroïds comedication		Ns		
Baseline Lymphocytes (x10⁶/L)	0.77 [0.58-1.02]	**0.0649**		Ns
Baseline NLR	1.05 [1.00-1.11]	**0.041**		Ns
Haemoglobin D21/D0		Ns		
Platelets D21/D0		Ns		
Lymphocytes D21/D0	1.72 [1.21-2.45]	**0.0028**	1.83 [1.24-2.71]	**0.0023**
Neutrophils D21/D0		Ns		
Grade ≥3 induced-lymphopenia		Ns		
No lymphopenia prior Sunitinib		Ns		
Composite AUC_{ss} at D21		Ns		

Composite AUC_{ss} is the sum of sunitinib and SU12662 at D21
Ns : not significant (univariate : p≥0.1; multivariate : p≥0.05)

2- Basal TK activity in PBMC from naïve mRCC patients

- Large Interindividual variability in patients' kinomic profiles (Figure 2).
- Phosphorylation level in PBMC from mRCC patients was lower than healthy volunteers for 74 peptides (p<0.05).

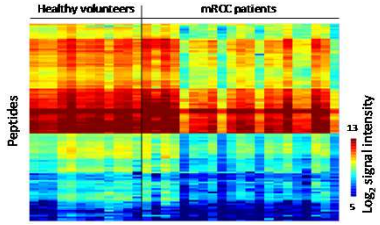

Figure 2 : Basal TK activity in PBMC lysates from healthy volunteers (n=12) and mRCC patients (n=21) sorted by hierarchical clustering,

3- *Ex vivo* sunitinib effect was associated with Heng prognostic model and D21/D0 lymphocytes ratio in patients

- Sunitinib and SU12662 decreased phosphorylation level for majority of peptides
- Sunitinib had a stronger inhibitory effect than SU12662 for 80 peptides (p<0.05).

A lower *ex vivo* sunitinib inhibition was significantly (p<0.05) correlated with :

- a poor prognosis according to Heng score (Figure 3A) for 53 peptides ,
- an increased D21/D0 lymphocytes ratio (Figure 3B) for 16 peptides .

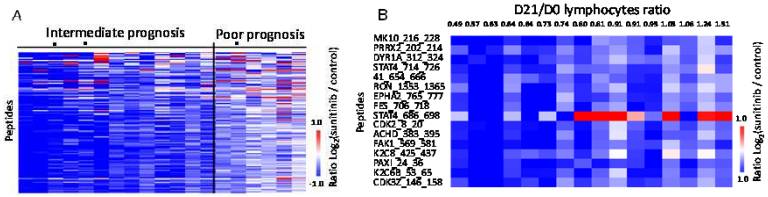

Figure 3 : Inhibition profiles obtained by addition of sunitinib (1 µM), sorted by column mean and rows, Correlation of *ex vivo* sunitinib inhibition profiles in PBMC from mRCC patients according to (A) Heng prognostic scores (n=19) and (B) lymphocytes ratio D21/D0 (n=16). *outliers

CONCLUSIONS

These preliminary results suggest that kinomic profiling of PBMC, could be a promising approach

- to investigate and decipher molecular mechanisms involved in sunitinib-induced immunomodulatory effects
- to seek future biomarkers

Further investigations are on going to determine the involvement of signaling pathways contributing to the inter-individual variability in kinomic profiles of PBMCs from mRCC patients treated with sunitinib.