Investigating the combination of bevacizumab and the EGF receptor inhibitor erlotinib for the treatment of metastatic renal cell carcinoma

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Background

- One of the major characteristics of metastatic clear cell renal cell carcinomas (RCC) is the over-expression of the Vascular Endothelial Growth Factor (VEGF). Several treatments against this pro-angiogenic factor are usually used such as sunitinib or the anti-VEGF bevacizumab (BVZ). In a mouse experimental model of RCC we have described that BVZ accelerated tumor growth. A down-regulation of phospho tyrosine phosphatase receptor kappa (PTPRk) by tumor cells under the selection pressure exerted during BVZ treatment is a potential explanation for this accelerated growth. The main target of PTPRk is the Epidermal Growth Factor Receptor (EGFR). Thus, down-regulation of PTPRk leads to a constitutive activation of the EGFR. According to the literature, BVZ combined with an inhibitor of EGFR (erlotinib/Tarceva) does not benefit patients. However, the EGFR mutations were not checked in this clinical study, contrary to what is done in lung cancer. Considering these data, a combination erlotinib (ERLO) with the BVZ/interferon alpha (INF) treatment may be more efficient against RCC growth.
- *Keywords : metastatic renal cell carcinoma - EGFR - bevacizumab - erlotinib - sunitinib*

Method

- Kidney tumors were operated at the Centre Hospitalier Princesse Grace (CHPG). Fuhrman grade of RCC was determined by CHPG Pathology Department. Cell Proliferation was measured by MTT assay 48h after drug exposure. For tumor growth studies, 786-O or A498 cells (3 million cells) were injected subcutaneously into the flank of 5-week-old nude (nu/nu) female mice (Janvier, France). Tumor volume was determined by using a caliper. BVZ (Avastin; Roche/Genentech, USA) was diluted with physiological serum and injected intra-peritoneally. Erlotinib (Tarceva, Roche) and was diluted with carboxymethylcellulose vehicle formulation and administered by oral gavage. BVZ mice were treated twice a week (7.5 mg/kg/injection) and received carboxymethylcellulose by oral gavage three times per week. Mice received Erlotinib treatment (50mg/kg) by oral gavage three times per week and physiological serum injection twice a week. mRNA expression of macrophages markers was determined by qPCR.

Results

- <u>New EGFR mutation in RCC cells</u>

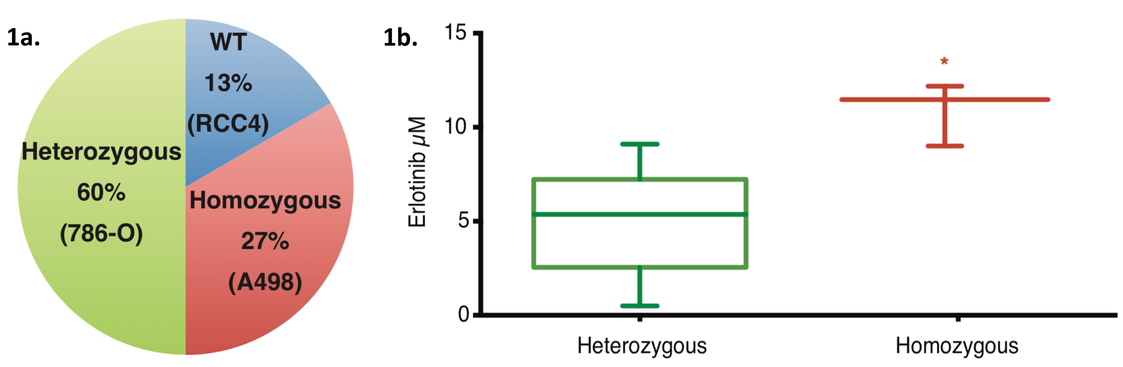
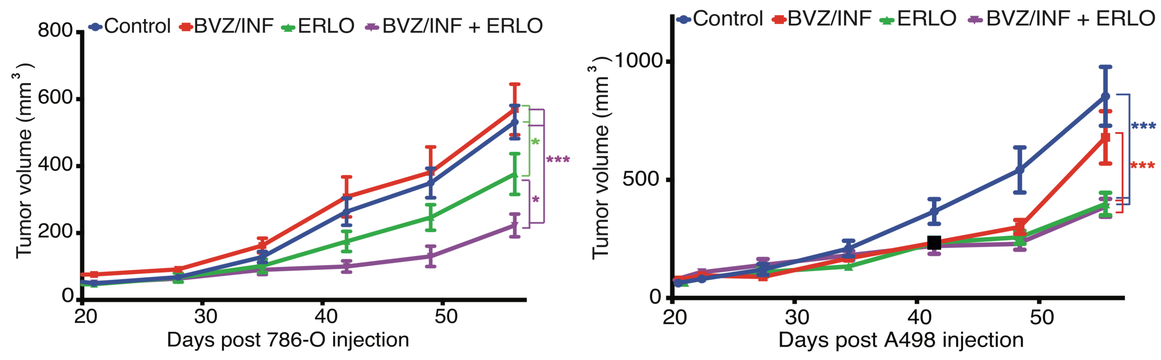


Figure 1 : This mutation may influence the sensitivity of RCC cells to erlotinib.
1a. A new mutation in the EGFR gene was identified in patient tumor cells. The number of heterozygous patients is superior to the homozygous ones, n = 15. The cells from the normal adjacent tissue are also mutated defining this mutation as germinal. ATCC cell lines are representative of the different possible mutation of status (RCC4 = WT; 786-O = heterozygous; A498 = homozygous).
1b. Sensitivity to erlotinib depending of the presence of the mutation. The IC50 was determined after 48 hours of treatment using MTT assay. The heterozygous cells are more sensitive to the treatment (IC50 = 5 µM) compared to the homozygous one (IC50 = 11 µM). Result is statistically significant (ttest p = 0.015).

- <u>EGFR expression in RCC cell lines</u>

Figure 2 : The mutation may affect the translational efficacy.
2a. EGFR in 786-O and A498 cells. A498 cells expressed more EGFR receptor than 786-O.
2b. Expression of EGFR wildtype (WT) or mutated (Mut) in HEK-293 cells. Non transfected (NT) cells expressed low level of this receptor. EGFR mutation induced a high amount of protein in cells.

- <u>Tumor xenograft growth treated with BVZ/INF + ERLO</u>

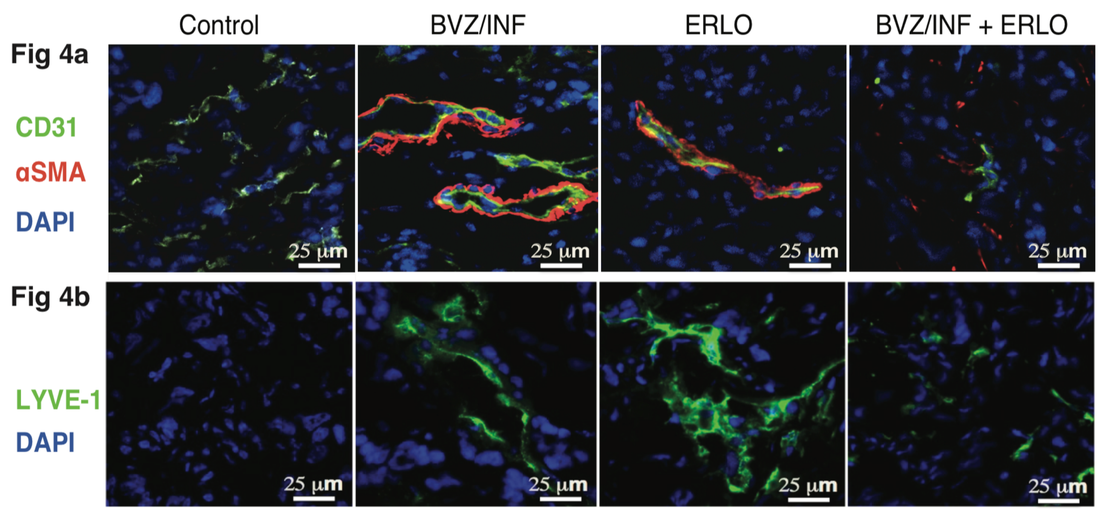


786-O xenograft are more sensitive to BVZ/INF + ERLO compared to ERLO alone or BVZ/INF. However, 786-O xenograft growth is reduced by ERLO compared to BVZ/INF treatment. One month after cells injection mice were treated.

A498 xenograft are senitive to ERLO treatment with ou without BVZ/INF. Tumor growth seems to escape to BVZ/INF treatment from the day 50. Mice were treated 15 days after cell injection.

<u>Figure 3</u> : Tumor growth of RCC cell xenograft treated with injection of BVZ/INF and/or ERLO. ERLO treatment is more efficient than BVZ/INF to reduce or delay 786-O and A498 tumor growth. Moreover, tumor generated with heterozygous cell line (786-O) are more sensitive to BVZ/INF + ERLO treatment.

- <u>Tumor vasculature after BVZ/INF and Erlotinib treatment</u>



<u>Figure 4</u> : 786-O tumor vascularization after BVZ/INF and/or erlotinib treatments.
4a. CD31 staining revealed the endothelial cells (green), pericyte are stained by alpha-SMA (red).
4b. Lymphatic vessels are stained by anti-LYVE-1 antibodies (green). Tumor sections are counterstained with DAPI .

- <u>mRNA expression of macrophages markers and alternative pro-angiogenic cytokines</u>

	Control	BVZ/INF	ERLO	BVZ/INF + ERLO
Macrophages M1 genes				
m iNOS	100	93	121	104
m IL6	100	110	73	127
Macrophages M2 genes				
m ARG1	100	8 (***)	334 (***)	19 (***)
m CD206	100	38 (***)	644 (***)	59 (*)
CXCL genes				
h CXCL5	100	100	309 (**)	49 (*)
h CXCL8	100	258 (**)	323 (**)	86

Figure 5 : mRNA gene expression from treated xenografts.
Mouse iNOS and IL6 genes are two markers of M1 macrophages polarization ARG1 and CD206 for M2 macrophages.
Human CXCL5 and CXCL8 genes are pro-angiogenic/ pro-inflammatory cytokines.

Conclusion

- We have identified a new mutation of the EGFR that could discriminate sensitive and insensitive patients to ERLO. BVZ/INF + ERLO treatment strongly reduced tumor growth and avoid tumor relapse as compared to the reference treatment sunitinib. BVZ/INF + ERLO prevents the development of a lymphatic network and decreases tumor vascularisation. The triple combination decreases the production of redundant pro-angiogenic/pro-inflammatory cytokines. This combinaison prevents the polarization of macrophages towards the M2 phenotype.

- Together, our results indicate that the BVZ/INF + ERLO combination is a relevant therapeutic strategy that deserves to be tested in patients mutated for the EGFR.

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