Preclinical studies on therapeutic targeting of FGFR2 fusion proteins: focus on intrahepatic cholangiocarcinoma

36

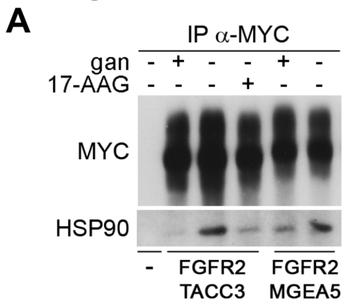
D. Lamberti[1] , G. Cristinziano[1] , M. Porru[2] , C. Leonetti[2] , I. Manni[2] , C. A. Amoreo[3] , S. Buglioni[3] , M. J. Borad[4] , S. Anastasi[1] and O. Segatto [1]

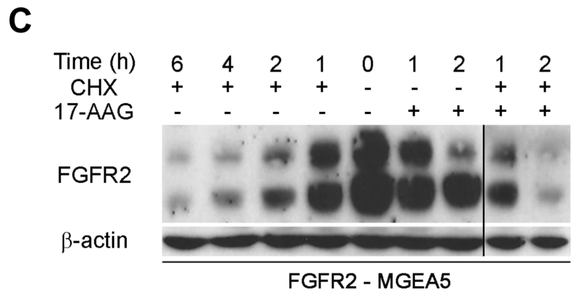
1 Unit of Oncogenomics and Epigenetics, IRCCS Regina Elena National Cancer Institute, Rome, Italy; 2 SAFU, IRCCS Regina Elena National Cancer Institute, Rome, Italy; 3 Department of Pathology, IRCCS Regina Elena National Cancer Institute, Rome, Italy; 4 Division of Hematology and Oncology, Mayo Clinic, Scottsdale, USA.

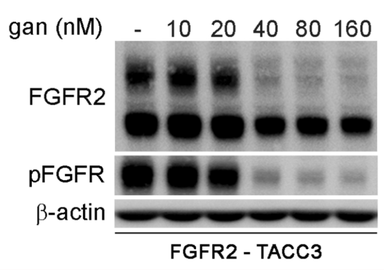
BACKGROUND About 15% of intrahepatic cholangiocarcinomas (ICC) express fibroblast growth factor receptor 2 (FGFR2) fusion proteins (FFs) generated by chromosomal translocations. FFs span aa. 1-762 of FGFR2IIIb joined C-terminally to sequences encoded by any of a long list of fusion genes (>30). FFs possess constitutive tyrosine kinase activity, which is caused by forced dimerization of the FGFR2 kinase domain imposed by protein-protein interaction motifs located in fusion sequences *(Wu YM et al., Cancer Discov, 2013).* FFs have been nominated ICC oncogenic drivers, based on clinical experimentation showing meaningful objective responses in FF-positive ICC patients treated with the FGFR tyrosine kinase inhibitor (F-TKI) BGJ398 *(Javle M et al., J Clin Oncol, 2017*).

AIM Our laboratory is interested in the pre-clinical development of novel therapeutic approaches to Intrahepatic Cholangiocarcinoma driven by FGFR2 fusion proteins

Figure 1: ICC FFs are Heat Shock Protein 90 (HSP90) clients

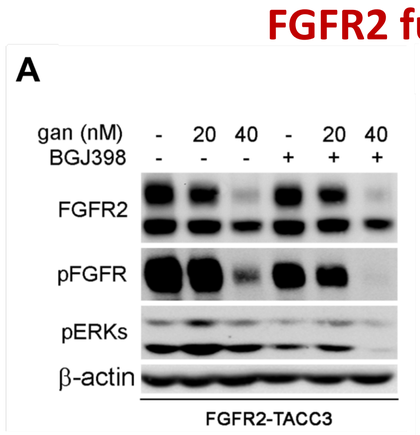


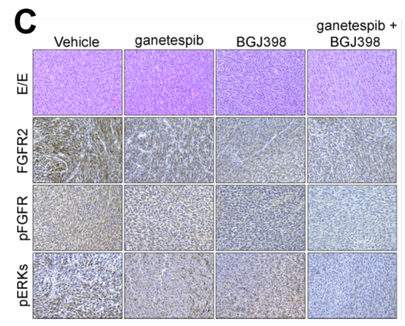


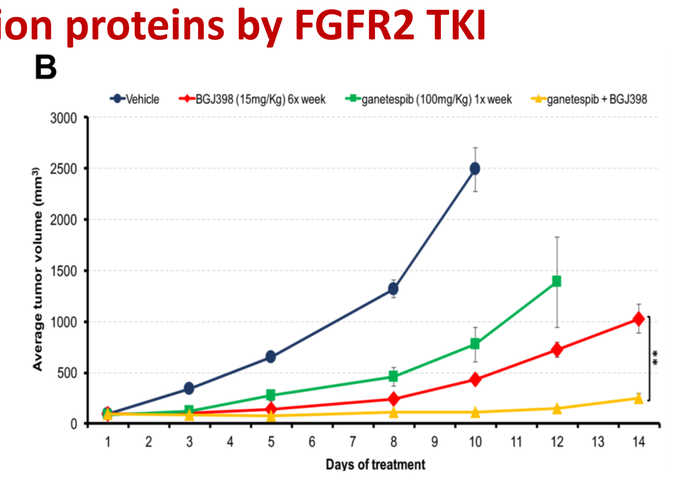


A) MYC tagged FGFR2–TACC3 and FGFR2-MGEA5 fusions are constitutively bound to HSP90 and this complex undergo rapid dissociation upon HSP90 inhibition by either 17-AAG or ganetespib. B) Ganetespib promotes proteolytic degradation of FGFR2-TACC3 fusion stably expressed in NIH-3T3 cells. C) CHX experiments indicate that, besides the mature higher MW form, also the immature species of FGFR2-MGEA5 undergoes swift degradation upon HSP90 inhibition. Similar results were obtained with all the three FFs analyzed.

Figure 2: HSP90 inhibition improves therapeutic targeting of FGFR2 fusion proteins by FGFR2 TKI

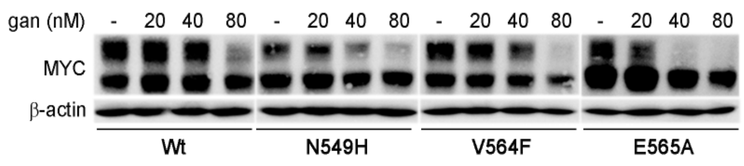


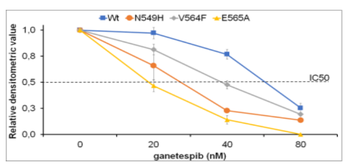




A) Combining FF catalytic suppression by BGJ398 with HSP90 blockade by ganetespib suppresses FGFR2-TACC3 signalling in cultured NIH-3T3 cells more effectively than single agents. B) The BGJ398 + ganetespib combo is superior to single agents when tested in mice carrying subcutaneous tumors generated by transplantation of FGFR2-TACC3 NIH-3T3 transformants. C) IHC of tumor sections indicates that the BGJ398 + ganetespib combo elicited the strongest inhibition of FGFR2-TACC3 oncogenic signaling.

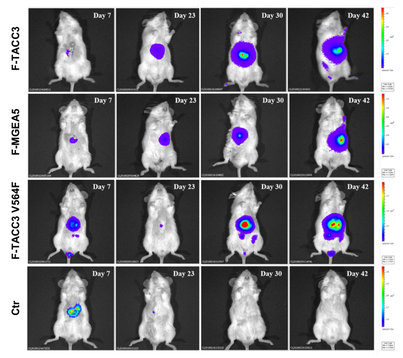
Figure 3: Clinically relevant BGJ398 resistant mutants retain sensitivity to ganetespib

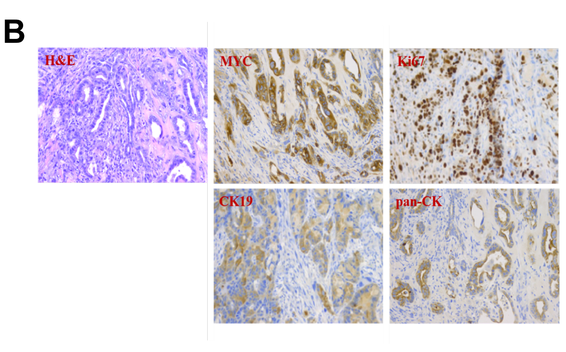




A major mechanism of clinical resistance to BGJ398 in ICC is the therapy induced selection of tumor subclones that are driven by mutations in the catalytic domain of FFs *(Goyal L et al., Cancer Discov, 2018).* FGFR2-TACC3 containing the BGJ398-resistant V564F "gatekeeper" mutation or the "molecular brake" N549H and E565A mutations retain sensitivity to ganetespib.

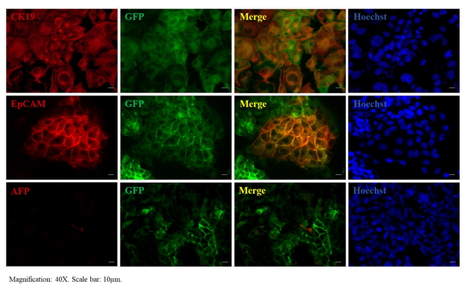
Figure 4: Generation of a mouse model of ICC driven by FGFR2 fusions

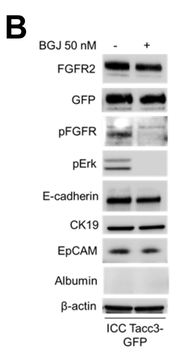


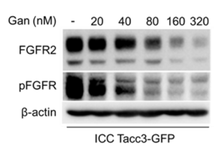


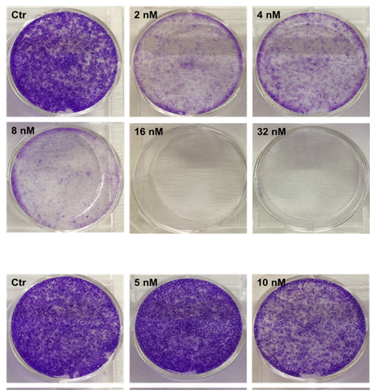
A) p53 null fetal hepatoblasts transduced with recombinant retroviruses encoding MYC tagged FGFR2-TACC3, FGFR2-MGEA5, FGFR2-TACC3 V564F gatekeeper mutant were injected in the liver of NOD-SCID mice. Controls were transduced with empty virus. Live imaging of luciferase activity shows progressive expansion of grafted cells. B) Representative histopathology of a liver lesion in a mouse injected with FGFR2-TACC3 hepatoblasts.

Figure 5: Oncogene addiction in a mouse-derived ICC cell line



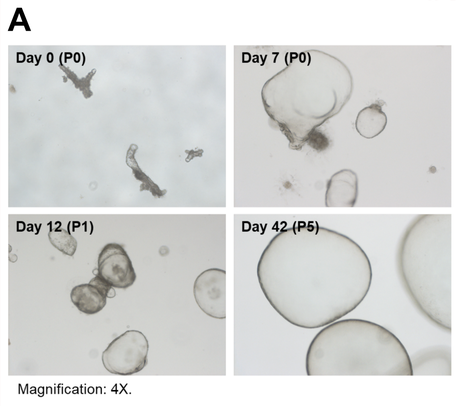


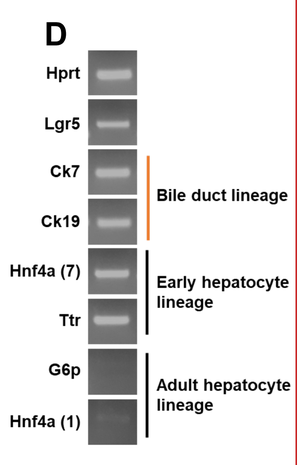




A, B) The ICC cell line expresses GFP-tagged FGFR2-TACC3, Ck19 and Epcam, but is negative for the hepatocyte markers Afp and Alb. Note inhibition of FF signaling by BGJ398 in panel B. C) Dose-dependent degradation of F-TACC3 in ICC1 cells treated with ganetespib. D) Dose-dependent inhibition of ICC cell proliferation by BGJ398 (upper panel) and ganetespib (lower panel)

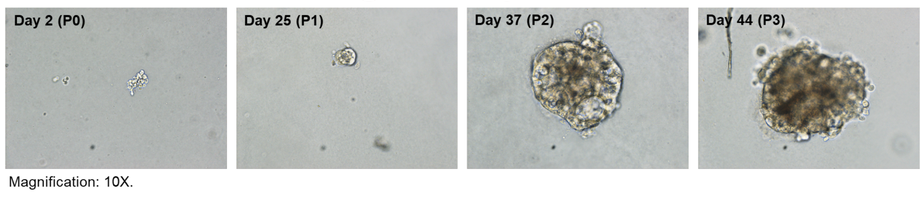
Figure 6: Liver organoids as a tool forgenetic modelling of ICC development?





A) Growth and passaging of organoids from adult mouse liver. B) Murine organoids transduced with a recombinant retrovirus encoding GFP C) Representative H&E staining of healthy liver derived organoids growing as a single-layered epithelium of ductal-like cells surrounding a central lumen. D) Analysis of cell lineage markers in murine liver organoids.

Figure 7: Generation of tumoroids from human ICC surgical explants



Magnification: 10X.

Growth and passaging of a human ICC tumoroid

Conclusions

Upfront treatment with the BGJ398 + ganetespib combo **a**) may improve therapeutic targeting of FGFR2 fusions; **b**) could be exploited to delay/prevent clinical resistance to BGJ398 caused by mutations in the FFs kinase domain *(Lamberti et al., HEPATOLOGY, 2018).*

Ongoing work

1) Our data suggest that FFs are sufficient to drive ICC development in a *Tp53* null background. **2**) We are implementing the mouse ICC tumoroid technology for in vitro studies on FF targeting. **3**) Tumoroids will also be used to derive secondary ICC lesions in recipient mice, thus representing a facile model for in vivo studies. **4**) We are building a collection of human ICC tumoroids to carry out in vitro and in vivo studies on genotype-matched therapeutic targeting of ICC. **5**) We are trying to use genetically engineered liver tumoroids for developing mouse ICC models recapitulating the human disease.

IRE ISTITUTO NAZIONALE TUMORI REGINA ELENA

15th Nov 2018

ISAB