FGFR selective inhibitor Debio 1347 induces tumor regressions in FGFR2-altered gastric cancer PDX models

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Abstract #4784

Summary

Dysregulation of the fibroblast growth factor receptor (FGFR) signaling pathway due to receptor over-expression, gene amplification, point mutations or fusions/chromosomal translocations is associated with cancer development and progression. This study was aiming at evaluating the impact of the type of FGFR alterations on the activity of Debio 1347 (CH5183284), an oral selective FGFR inhibitor currently in clinical development.

A mouse trial was conducted in 39 different PDX models of various histotypes selected according to their FGFR1, 2 and 3 alteration status including gene amplification, expression and fusion. Debio 1347 was administered orally once daily and tumor volume was monitored for 14 consecutive days.

In gastric cancer, Debio 1347 induced tumor regression in models harboring the commonly found FGFR2 amplification. Interestingly, Debio 1347 was also highly effective in a model with high FGFR2 expression but without FGFR2 amplification, indicating that not only gene amplification drives the activity of Debio 1347 in this indication, but also the expression level of FGFR2.

Altogether, these data demonstrate that Debio 1347 could be highly effective in gastric cancer patients harboring FGFR2 amplification and/or high FGFR2 expression. Debio 1347 is currently investigated in a Phase I trial in selected patients harboring FGFR alterations (NCT01948297).

Background

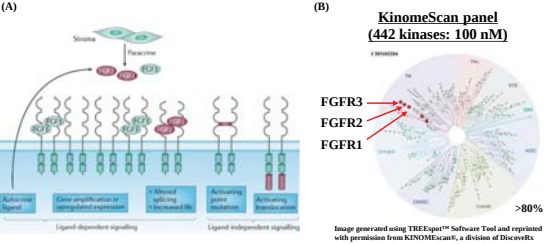

Figure 1. (A) Mechanisms of FGFR activation (adapted from Knowles et al., 2015[1]). **(B)** TREEspot™ Interaction Map for Debio 1347 tested at 100 nM[2] (Kinome Scan panel, DiscoveRx).

Methods

PDX mouse models (CrownBio, China). Studies were approved by the local Ethics Committee for Animal Experimentation. Briefly, tumor fragments (2-3 mm in diameter) were injected subcutaneously (sc) into the right flank of female Balb/c nude mice. Tumor volume was measured twice weekly in two dimensions using a caliper, and the volume was expressed in mm^3 using the formula: $V = 0.5 \times b^2$ where a and b are the long and short diameters of the tumor, respectively. Body weight was also recorded twice weekly. Debio 1347 was orally administered once a day for 14 consecutive days in mice with established tumors (n=3 vehicle controls and n=3 treated animals).

FISH. Hybridization to an FGFR probe was performed as previously described[3]. FGFR probes used were FGFR1/CEN 8 Dual Color Probe, FGFR2/CEN 10 Dual Color Probe and FGFR3/4p11 Dual Color Probe (all from Zytovision GmbH, Bremerhaven, Germany). Gene copy number gain (GCN) was defined as either amplification (FISH FGFR probe-centromere probe ratio ≥2.2) or polysomy, defined as FGFR probe-centromere probe ratio <2.2 but each one of FGFR and centromeres probes >2.

Immunohistochemistry (IHC). Immunostaining was performed on 4-µm paraffin-embedded tissue sections using standard protocol. FGFR2 Rabbit polyclonal antibody was from Novus Biologicals (Cat# NB200-642).

mRNA expression analyses. Total RNA from FFPE tissue macrodissected sections was isolated using the miRNeasy FFPE kit (QIAgen). For NanoString analysis, 300 ng of total RNA per sample were analyzed using the nCounter Gene Expression Assay protocol as instructed by the manufacturer. For qPCR, 500 ng of total RNA were reverse-transcribed using the Transcriptor Universal cDNA Master (Roche), and the resulting cDNA was amplified using FAM dye–labeled TaqMan Assays (Roche, Thermo) and the LightCycler® 1536 DNA Probes Master (Roche). Counts and relative quantities normalization were performed using the most stably expressed reference genes validated using the geNorm algorithm[4].

References

(1) Knowles MA et al. Molecular biology of bladder cancer: new insights into pathogenesis and clinical diversity. Nat Rev Cancer. 2015 Jan;15(1):25-41.
(2) Nakanishi Y et al. FGFR genetic alterations as a potential predictor of the sensitivity to CH5183284/Debio 1347, a selective FGFR inhibitor with a novel chemical scaffold. AACR 2014 abstract #2729.
(3) Schildhaus, H.U. et al. Definition of a fluorescence in-situ hybridization score identifies high- and low-level FGFR1 amplification types in squamous cell lung cancer. (2012) Mol. Pathol. 25: 1473-80.
(4) Vandesompele J et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 2002 Jun 18;3(7):research0034.1–research0034.11.

Results

Debio 1347 demonstrates high anti-tumoral efficacy in PDX models of different histotypes

39 PDX models of different histotypes representing the four following categories were selected and tested for efficacy : (i) increased copy number of an FGFR gene and overexpression of an FGFR, (ii) increased copy number of an FGFR gene without overexpression of an FGFR, (iii) no increase in copy number of an FGFR gene but overexpression of an FGFR, and (iv) expression of an FGFR fusion gene product.

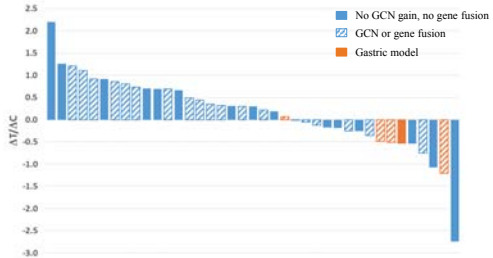

Figure 2. Waterfall plot of the ΔT/ΔC values of all 39 PDX models. Treatment efficacy was expressed as ΔT/ΔC, whereby ΔT reports the relative change in tumor volume of drug-treated animals and ΔC the relative change in tumor volume of not-drug-treated animals between the last day of treatment and the beginning of treatment (median volume differences). ΔT/ΔC <0 corresponds to complete arrest of tumor growth during the treatment period.

Debio 1347 induced strong anti-tumoral efficacy in PDX models of different histotypes (Fig. 2). Efficacy was particularly noticed in Gastric models. Therefore, we focused the rest of the work presented in this poster on gastric models.

Debio 1347 induces tumor regressions in gastric PDX models

A total of 11 PDX models of gastric origin were investigated. Representative models with no FGFR2 alterations (Fig 3A), FGFR2 high mRNA expression but no amplification (Fig 3C) or FGFR2 high mRNA expression and amplification (Fig 3E) show differential sensitivity profiles to Debio 1347:

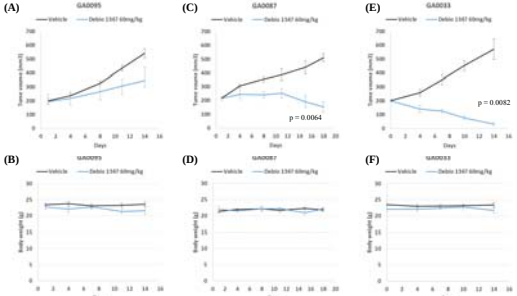

Figure 3. Response to Debio 1347 in representative gastric PDX models. Debio 1347 was administered orally daily at 60 mg/kg for 14 **(A, E)** or 18 **(C)** consecutive days. Debio 1347 was very well tolerated as measured by body weight **(B, D, F)**. Debio1347 resulted in a statistically significant reduction in mean tumor volume when compared to the vehicle group (unpaired two-tailed t-test).

Characterization of FGFR2 amplification and expression level by different technologies

Models were characterized by FISH and IHC for FGFR2. As none of the commercial antibody tested for FGFR2 gave reliable results, we further focused on mRNA levels by using either RT-qPCR or Nanostring technologies.

MODEL ID	ΔT/ΔC	FGFR2 GCN
16	0.0692	polysomy
17	-0.5356	normal
18	-0.4864	amplified
19	-0.5062	amplified
20	-1.2121	amplified
50	0.4235	polysomy
51	0.9224	normal
52	0.8812	polysomy
53	0.3978	normal
54	-0.5015	amplified
55	0.8524	polysomy

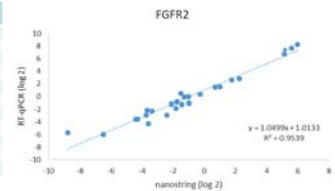

Figure 4. mRNA levels measured by each of qRT-PCR and NanoString technologies on test sets of 26 PDX gastric tumor samples.

A very good correlation is observed between the two tested technologies, confirming the robustness of the Nanostring technology for further assessments.

Debio 1347 induces tumor growth delay and necrosis

Debio 1347 administered at 30mg/kg induced a significant tumor growth inhibition without inducing tumor regressions, whereas increasing the dose up to 60mg/kg did induce tumor regressions.

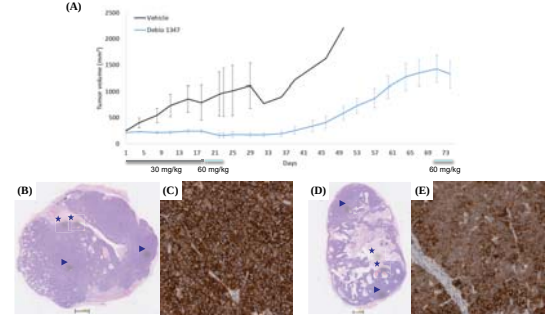

Figure 5. Efficacy in GA1224 PDX model. Debio 1347 was administered at 30 mg/kg from D1- D18, then dose level was increased from D19 to D22 to 60 mg/kg. After treatment free period, animals received 60mg/kg treatment from D68 to D74 **(A)**. Representative H&E staining after 23 days **(B)** and on Day 74 **(D)**, scale = 1mm, stars: necrotic area, arrowheads: tumoral area. FGFR2 IHC staining of Debio 1347-treated animal at D23 **(C)** and Day 74 **(E)**.

Debio 1347 induced significant tumor growth delay. Debio 1347 triggered tumor necrosis (Fig 5B, stars) which increased over time even after treatment withdrawal (Fig 2D).
Even though tumors started to re-grow after treatment was stopped (on Day 23), tumors were still sensitive to Debio 1347 (administered at 60mg/kg from Day 68 to Day 74). This sensitivity to FGFR inhibition is supported by the persistence of FGFR2 protein expression level within tumor (Fig 5C and 5E).

FGFR2 expression level can classify models sensitive to Debio 1347

An increased level of FGFR2 was associated with better anti-tumoral efficacy of Debio 1347 (Spearman correlation p-value: 0.015).

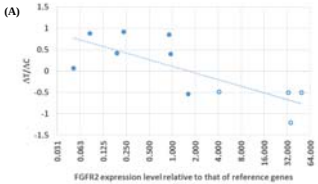

Figure 6. Relationship between response to Debio 1347 (ΔT/ΔC) and FGFR2 mRNA expression. (A) Same models were clustered into the 2 following categories : responding models (ΔT/ΔC<0) and non-responding models (ΔT/ΔC>0) in function of FGFR2 mRNA expression levels, while also indicating FGFR2 amplification (FISH ratio > 2.2, empty circles) **(B).**

Responding models and non-responding models clearly displayed different levels of FGFR2 expression. Selecting models on the basis of FGFR2 amplification only (empty circles) does not allow to select all responding models. Indeed, one model displaying no amplification but high level of FGFR2 expression responded to Debio 1347.

Conclusions

- **Debio 1347 demonstrated high anti-tumoral efficacy in PDX models of different histotypes.**
- **In gastric cancer, Debio 1347 induced tumor regression in models harboring the commonly found FGFR2 amplification.**
- **Interestingly, Debio 1347 was highly effective in model with high FGFR2 expression but without FGFR2 amplification, indicating that not only gene amplification drives the activity of Debio 1347, but also gene expression.**
- **Debio 1347 is currently investigated in a Phase I trial in selected patients harboring FGFR alterations (NCT01948297).**

Related Presentations

- ERK signal suppression and sensitivity to CH5183284/Debio 1347, a selective FGFR inhibitor. Sunday, Apr 19, 8:00 AM - 12:00 PM- Abstract #3028

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