# Mechanism of oncogenic signal activation by the novel fusion kinase FGFR3-BAIAP2L1

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## SUMMARY

Recent cancer genome profiling studies have identified many novel genetic alterations, including rearrangements of genes encoding fibroblast growth factor receptor (FGFR) family members. However, most fusion genes are not functionally characterized, and their potentials in targeted therapy are unclear. In a previous study (1), we investigated the recently discovered gene fusion between *FGFR3* and BAI1-associated protein 2-like 1 (*BAIAP2L1*). We identified 4 patients with bladder cancer and 2 with lung cancer harboring the fusion gene via screens involving PCR and a break-apart fluorescence *in situ* hybridization assay. To understand the functional roles of this fusion gene in tumors, we established an *FGFR3-BAIAP2L1* transfectant in Rat-2 fibroblast cells (Rat-2_F3-B). The *FGFR3-BAIAP2L1* fusion had transforming activity in Rat2 cells, and Rat-2_F3-B cells were highly tumorigenic in mice. Rat-2_F3-B cells showed *in vitro* or *in vivo* sensitivity to CH5183284/Debio 1347 (2)*, a selective FGFR inhibitor, indicating that the FGFR3 kinase activity is critical for tumorigenic activity. We also established Rat-2_F3-B-ΔBAR cells, which expressed a FGFR3-BAIAP2L1 variant lacking the Bin–Amphiphysin–Rvs (BAR) dimerization domain of BAIAP2L1 and exhibited decreased tumorigenic activity and FGFR3 phosphorylation compared to Rat-2_F3-B cells. Diminished dimerization was observed with the F3-B-ΔBAR protein compared with FGFR3-BAIAP2L1. Collectively, these data suggested that constitutive dimerization though the BAR domain promotes constitutive FGFR3 kinase activation and is essential for its potent oncogenic activity. In the present study, we investigated the signaling pathway of FGFR3-BAIAP2L1 more profoundly. We conducted a comprehensive gene expression analysis by NGS using 4 cell lines (Rat-2_mock, Rat-2_FGFR3, Rat-2_F3-B, and Rat-2_BAIAP2L1) and identified 143 up-regulated genes and 67 down-regulated genes specifically engaged by FGFR3-BAIAP2L1. Gene signature analysis with the gene set revealed that FGFR3-BAIAP2L1 activates growth signals, such as the mitogen-activated protein kinase pathway, and inhibits tumor-suppressive signals, such as the p53, RB1, and CDKN2A pathways. Then, we confirmed those pathway activation and inactivation with western blotting in xenograft tissue. These data suggested that a concurrent regulation of an oncogenic pathway and a tumor-suppressive pathway could be a potential tumorigenic mechanism of FGFR3-BAIAP2L1.

## METHODS

**RT-PCR:** The cDNAs were obtained from OriGene Technologies, inc.. PCR was carried out (42 cycles of 10 seconds at 94°C, 15 seconds at 55°C, and one minute at 68°C) with Tks Gflex DNA Polymerase (Takara bio) using, as primers, oligonucleotides having the nucleotide sequences of 5'-TGTTTGACCGAGTCTACACTCACC-3' and 5'-GACATGTCCCAGTTCAGTTGAC-3'.

**Cell proliferation assay:** Cell lines were obtained from ATCC, DSMZ, HPACC, JCRB, and HSRRB. All cell lines were cultured according to supplier instructions. The cell lines were added to the wells containing 0.0030–20,000 nM CH5183284/Debio 1347 and incubated at 37°C. After 4 days' incubation, the viable cells were measured by the WST8 (DOJINDO).

**Mouse xenograft study:** All *in vivo* studies were approved by the Chugai Institutional Animal Care and Use Committee. Female BALB-nu/nu mice were obtained from Charles River Laboratories Japan. Cells were suspended in serum-free culture medium and injected subcutaneously into the right flank of the mice. Tumor size was measured using a gauge twice per week, and tumor volume (TV) was calculated using the following formula: $TV = ab^2/2$, where a is the length of the tumor, and b is the width. CH5183284/Debio 1347 was orally administered once a day in established tumors.

**Western blot analysis:** Cells were lysed with Cell Lysis Buffer (Cell Signaling Technology) containing protease and phosphatase inhibitors. The grafted tumors were homogenized using a BioMasher (K.K. Ashisuto) before lysis. The lysates were denatured with Sample Buffer Solution with Reducing Reagent for SDS-PAGE (Life Technologies) and were then subjected to SDS-PAGE. After electroblotting, western blot analysis was performed by conventional methods.

**RNA-Seq and expression analysis**: Cellular RNA was extracted using the RNeasy Mini Kit (Qiagen, Inc.). Quality assessment, poly-A selection, and sequencing with a HiSeq 2000 Sequencing System (Illumina) were performed by Macrogen, Inc. Cellular RNA samples were prepared for sequencing using a TruSeq RNA Sample Preparation Kit (Illumina) to generate an mRNA library, and 100 bases were sequenced from both ends of the library. RSEM software was used to align reads against RefSeq transcripts and calculate expression values for each gene. Fold-changes in expression levels were calculated to identify down-regulated genes (<80% expression) and up-regulated genes (>120% expression), relative to Rat-2_mock cells and other cell lines. We also purified and sequenced total RNA from Rat-2_F3-B cells treated for 24 h with either 0.1% DMSO or 1 µmol/L CH5183284/Debio 1347. Fold-changes were calculated by normalizing gene expression levels in CH5183284/Debio 1347-treated cells to DMSO control cells, identifying suppressed (< 50% expression) or induced genes (>200% expression), relative to DMSO controls.

## RESULTS

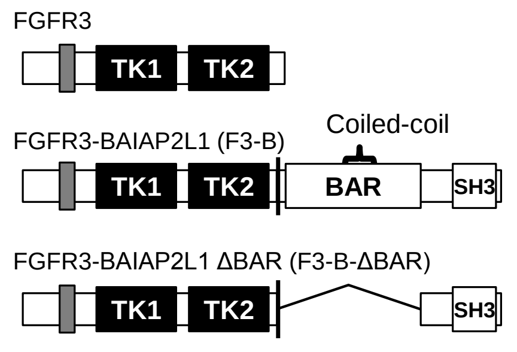
### Oncogenic potential of FGFR3-BAIAP2L1

**Prevalence of FGFR3-BAIAP2L1 in patients**

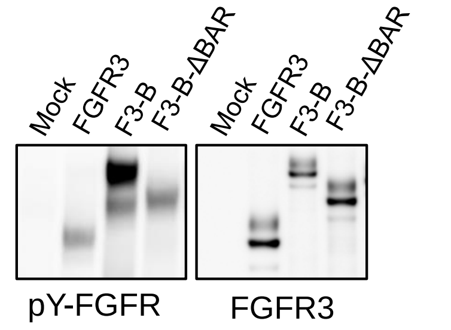
| Tumor type | | N (%) |
|---|---|---|
| Lung cancer | Adenocarcinoma (n = 28) | 1 (3.6) |
| | Squamous cell (n = 28) | 1 (3.6) |
| | Others (n = 27) | 0 (0) |
| Bladder cancer (n = 46) | | 2 (4.3) |
| Head & neck cancer (n = 17) | | 0 (0) |
| Gastroesophageal cancer (n=18) | | 0 (0) |

Detection by RT-PCR

**Schematic diagrams**

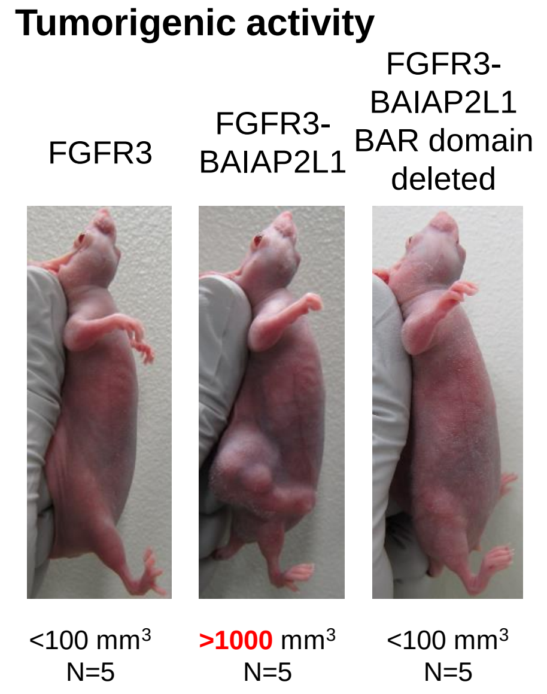


**FGFR activation by BAR domain**



pY-FGFR FGFR3

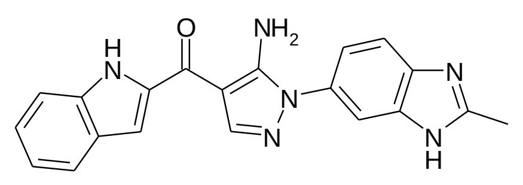
**Tumorigenic activity**



Rat-2 stable cell lines

### Potent anti-tumor activity of CH5183284/Debio 1347, a FGFR selective inhibitor against FGFR3-BAIAP2L1 tumors

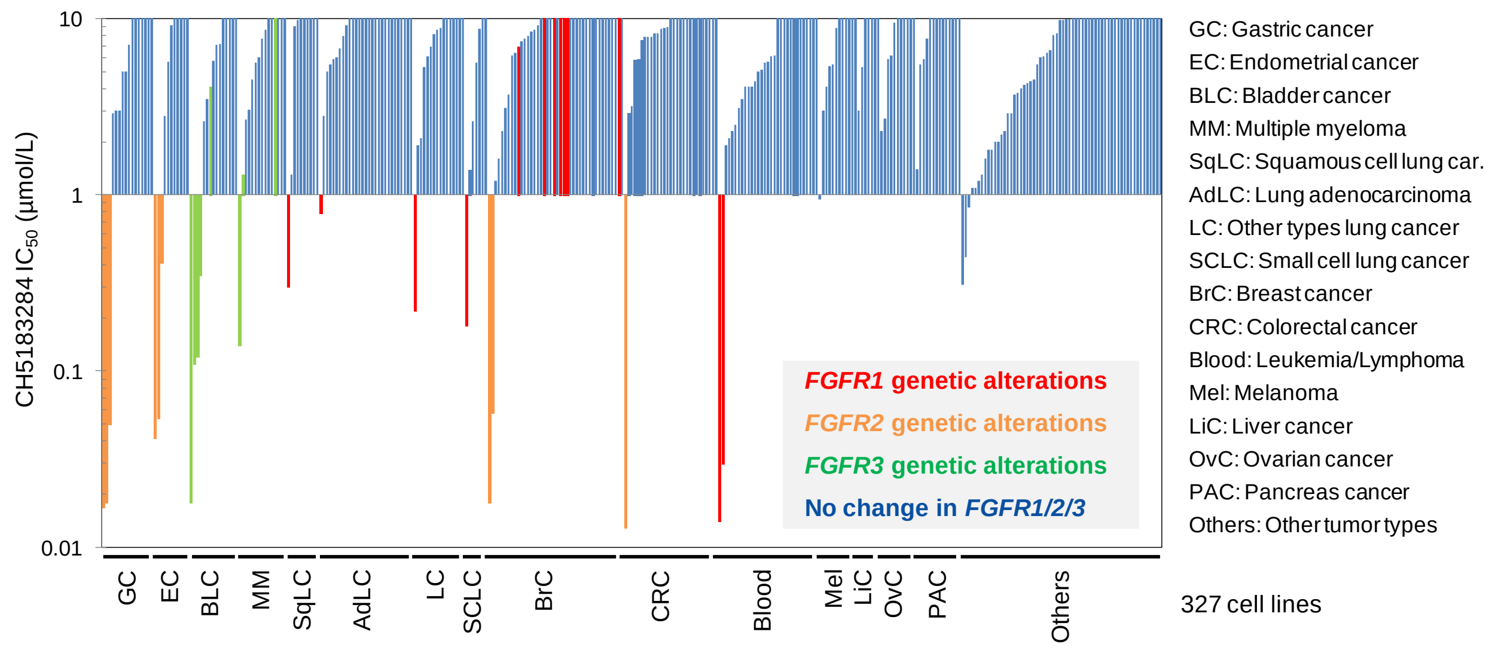
**Chemical structure of CH5183284/Debio 1347**



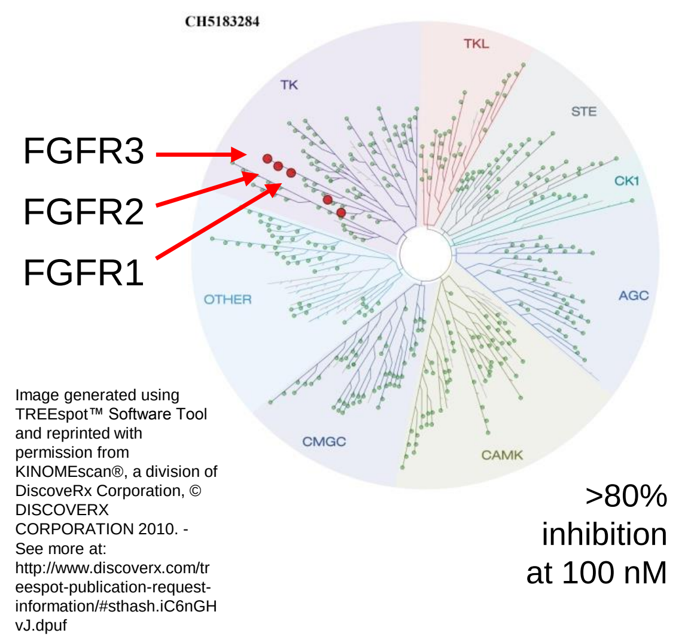
ATP competitive
Kinase activity $IC_{50}$ (nM)

| | |
|---|---|
| FGFR1 | 9.3 |
| FGFR2 | 7.6 |
| FGFR3 | 22 |
| FGFR4 | 290 |
| KDR | 2100 |

**Selective *in vitro* anti-proliferative activity against FGFR genetically altered cancer cell lines**



**Selective kinase inhibitory profile In KinomeScan panel (442 kinases: 100 nM)**



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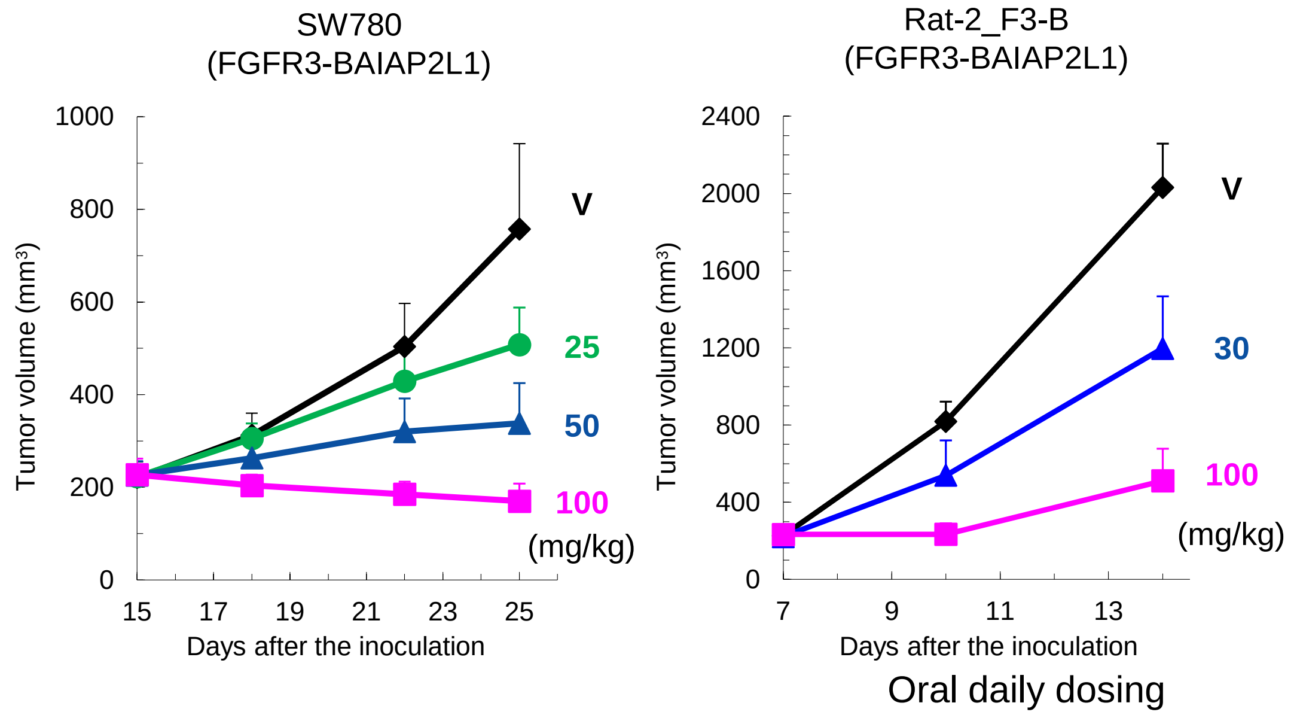
**Antitumor activity of CH5183284/Debio 1347 in xenograft mouse models harboring FGFR3-BAIAP2L1**



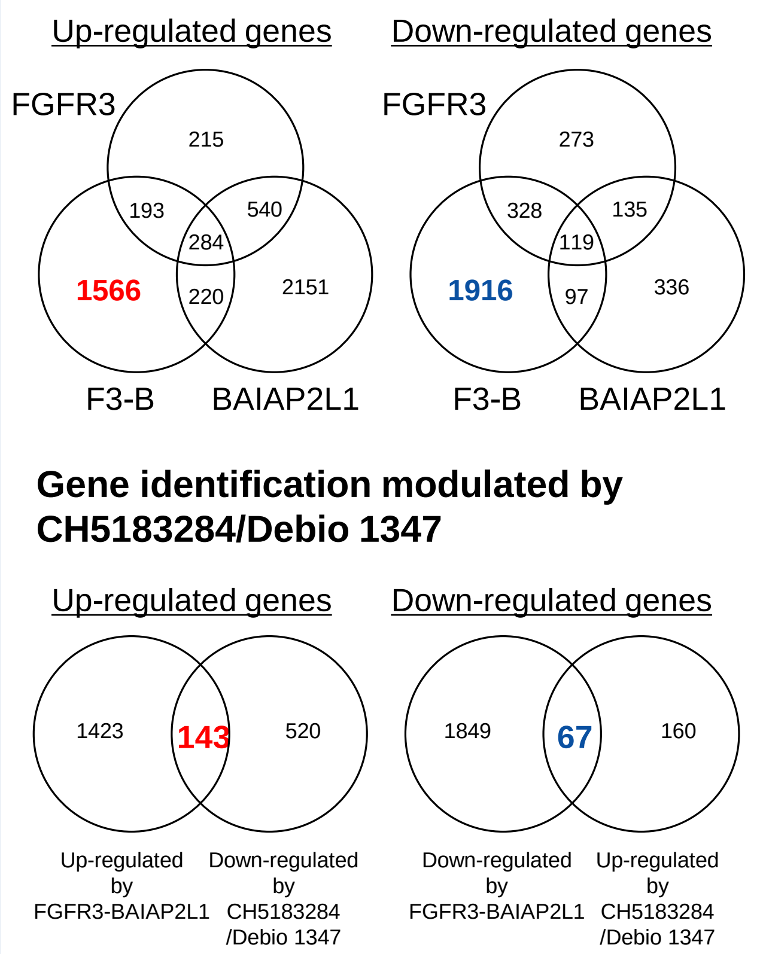
### Scheme of pathway analysis

Purification of total RNA at the normal culture condition of Rat-2 stable cell lines
↓
mRNA expression analysis with RNAseq
↓
Identification of FGFR3-BAIAP2L1 signature
↓ *1,566 upregulated genes*
*1,916 downregulated genes*
Purification of total RNA Rat-2 stable cell lines treated with CH5183284/Debio 1347
↓
Identification of genes modulated by CH1583284/Debio 1347 treatment from FGFR3-BAIAP2L1 signature
↓ *143 upregulated genes*
*67 downregulated genes*
Apply the gene set to Ingenuity pathway analysis
↓
Confirmation of observations with western blot

### Pathway identification regulated by FGFR3-BAIAP2L1

**FGFR3-BAIAP2L1 signature finding**

**Gene identification modulated by CH5183284/Debio 1347**

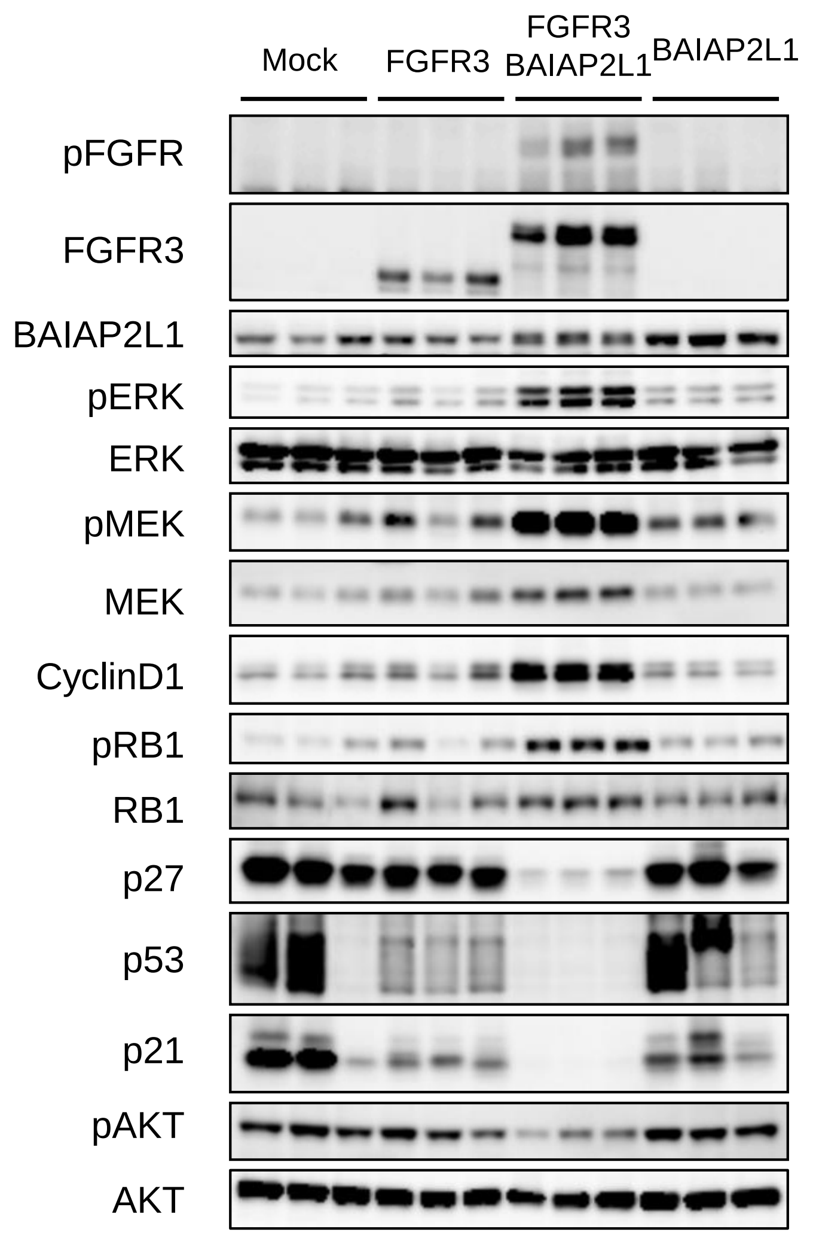


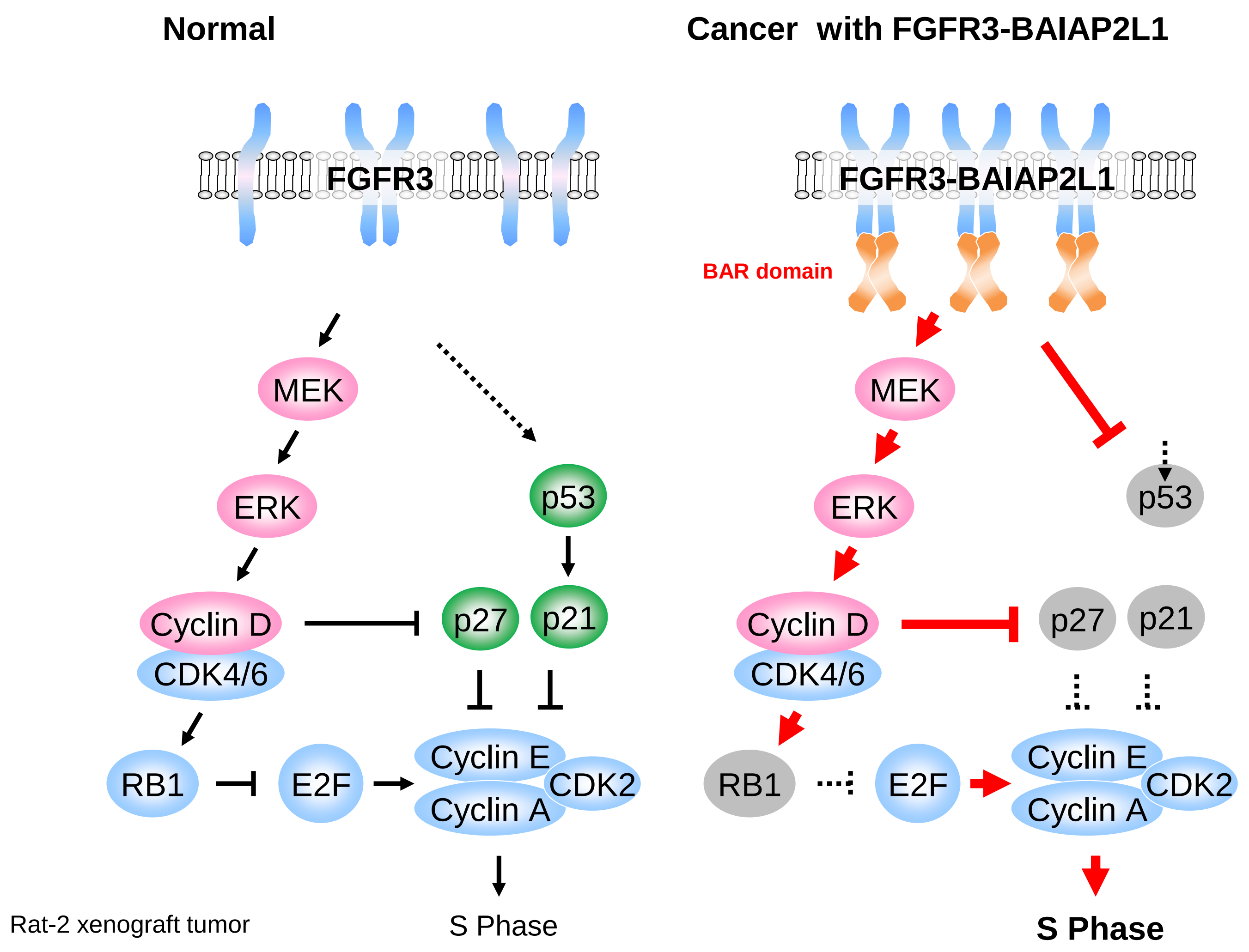
**Ingenuity pathway analysis**

| Upstream regulator | Predicted state in Rat-2_F3-B | Activation z-score | p-value of overlap |
|---|---|---|---|
| RB1 | Inhibited | -2.95 | 9.5E-17 |
| TP53 | Inhibited | -2.05 | 1.4E-12 |
| RBL1 | Inhibited | -2.75 | 1.1E-11 |
| CDKN2A | Inhibited | -2.31 | 4.9E-10 |
| NUPR1 | Inhibited | -3.21 | 2.2E-06 |
| E2F2 | Activated | 2.00 | 1.4E-13 |
| E2F1 | Activated | 3.52 | 1.3E-12 |
| TBX2 | Activated | 2.83 | 1.9E-08 |
| CEBPB | Activated | 2.36 | 1.9E-05 |
| STAT3 | Activated | 2.40 | 2.7E-04 |

### Significant activation of MAPK pathway and suppression of tumor suppressive pathway by FGFR3-BAIAP2L1

**Signal modulation in Rat-2 xenograft tumor**





## CONCLUSION

- **FGFR3-BAIAP2L1 fusion was identified in patients and showed potent tumorigenic potential activated by dimerization via the BAR domain of BAIAP2L1.**
- **The selective orally available FGFR inhibitor, CH5183284/Debio 1347, effectively inhibits *in vivo* tumor growth of cells harboring FGFR3-BAIAP2L1.**
- **FGFR3-BAIAP2L1 could activate MAPK pathway and attenuate tumor suppressive pathways. (ex. TP53)**

In summary, treating patients harboring FGFR gene fusions such as FGFR3-BAIAP2L1 with CH5183284/Debio 1347 could be a promising approach in the future. Also, according to the pathway analysis, a combination therapy with MAPK pathway inhibitor would be considered as a vertical pathway inhibition approach.

## CLINICAL TRIAL

* Debio 1347/CH5183284 is currently under phase I clinical investigation by Debiopharm International S.A. in selected patients harboring FGFR genetic alterations(NCT01948297).

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## RELATED PRESENTATIONS

• #639, Sunday Apr 19, 2015 1:00 PM - 5:00 PM
Characterization of two novel oncogenic FGFR2 fusions sensitive to the FGFR-selective inhibitor Debio 1347 in cholangiocarcinoma
. CT228, Monday, April 20, 2015, 8:00 AM – 12:00 AM
Formulation switch and pharmacokinetics/pharmacodynamics of Debio 1347 (CH5183284), a novel FGFR inhibitor, in a first-in-human dose escalation trial in solid tumors patients

## REFERENCES

1. Mechanism of oncogenic signal activation by the novel fusion kinase FGFR3-BAIAP2L1. Nakanishi Y., Akiyama N., et al, Mol Cancer Ther. 2015; 14(3): 704-712
2. The fibroblast growth factor receptor genetic status as a potential predictor of the sensitivity to CH5183284/Debio 1347, a novel selective FGFR inhibitor. Nakanishi Y., et al, Mol Cancer Ther. 2014;13(11):2547-58.