ODM-203, a novel, selective and balanced FGFR and VEGFR inhibitor with strong activity on primary and metastatic tumor growth in FGFR- and VEGFR-dependent cancer models

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Building well-being

Background

Genomic alterations in fibroblast growth factor receptors (FGFR) and upregulation of vascular endothelial growth factor receptors (VEGFR) are often found in the same cancer types such as bladder, breast, gastric, and lung and these alterations correlate with patient survival and disease progression. In addition, both FGFR and VEGFR signaling promote tumor angiogenesis. Activation of FGFR signaling has also been described to function as a compensatory angiogenic signal following development of resistance to VEGF inhibition.

Methods

In vitro kinase assays
The selectivity of the compounds was tested against 317 wild type protein kinases at 1µM concentration with total 1µM ^{33}P-ATP. Kinase inhibition IC50 values were determined for seven kinases with a final concentration of 10µM ATP and ^{33}P-ATP mix.

Cell proliferation assays
H1851, SNU-16 and RT-4 cell lines were obtained from ATCC. H1581 is a lung cancer cell line with FGFR1 amplification, SNU16 is a gastric cancer cell line with FGFR2 amplification and RT4 is a bladder cancer cell line with FGFR3 translocation. Cell proliferation was measured by using WST-1 Cell Proliferation Assay (Roche) and Envision microplate reader (Perkin Elmer).

Cell-based angiogenesis assay
The ability of the test compounds to inhibit angiogenesis was studied in the VEGF-driven tube formation assay using the GFP-AngioKit Cell Player co-culture model (Essen Biosciences).Test compounds were added in the presence of VEGF from day 2. Vascular tube formation was monitored using an IncuCyte-FLR live cell imaging system (Essen Biosciences).

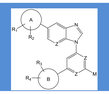
Immunoblotting
HUVEC cells (Life Sciences) were serum starved with 1% FBS o/n, treated for 1h with ODM-203 and 20 ng/ml VEGF (Sigma) was added for 10 min. SNU-16 cell line (ATCC) was treated for 1h with ODM-203. HUVEC samples were immunoblotted with pVEGFR2 (Cell Signaling) and VEGFR2 (R&D Systems), SNU-16 samples with pFGFR (Cell Signaling) and FGFR2 (Abcam) antibodies.

Subcutaneous xenograft models
Tumors were established by subcutaneous injection of RT4 or SNU-16 cells into male nude mice. Oral treatment (ODM-203, or Selective FGFRi) was initiated when the average tumor volume reached ~125 mm3. Mean tumor volumes were calculated for each treatment group.

Orthotopic Renca xenograft model
Tumors were established by injection of Renca cells under the kidney capsule of male nude mice. Oral treatment (ODM-203, or Selective FGFRi) was initiated two days after injection of cells. Mean tumor weight was measured after 21 days for each treatment group. Lung nodules were counted upon terminal necropsy.

Results

1. ODM-203 is selective and equally potent against FGFR and VEGFR family kinases



IC50 (nM)	ODM-203	Lucitanib	Selective FGFRi
FGFR1	11	58	0.3
FGFR2	16	186	0.2
FGFR3	6	253	1
FGFR4	35	> 1000	7
VEGFR1	26	162	87
VEGFR2	9	9	55
VEGFR3	5	34	35

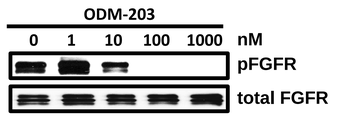
In addition to the above mentioned kinases ODM-203 inhibits only 9 kinases out of 317 to > 70% at 1 uM

2. ODM-203 shows similar potency in inhibiting growth of FGFR dependent cell lines and angiogenesis

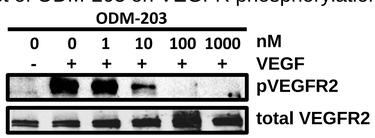
Cell line (receptor) / IC50 nM	ODM-203	Lucitanib	Selective FGFRi
H1581 (FGFR1)	104	160	6
SNU 16 (FGFR2)	132	65	5
RT4 (FGFR3)	192	130	21
Angiogenesis (tube formation)	33	1	260

3. ODM-203 is equally potent in suppressing FGFR and VEGFR dependent signalling in cells

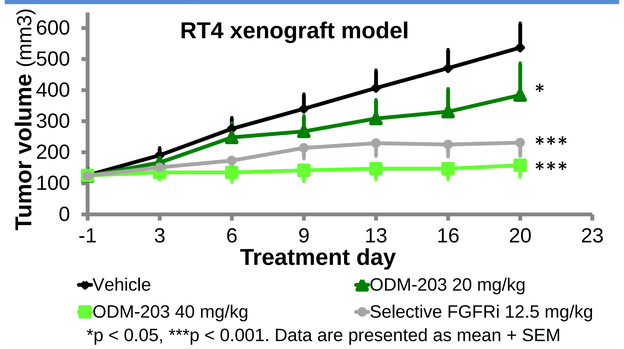
A. Effect of ODM-203 on FGFR phosphorylation in a FGFR dependent cell line (SNU16)



B. Effect of ODM-203 on VEGFR phosphorylation in HUVEC cells

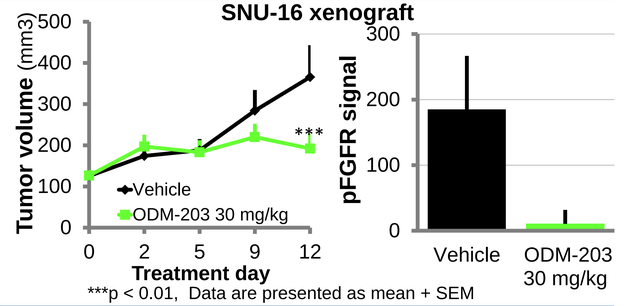


4. ODM-203 shows strong anti-tumor activity in FGFR dependent xenograft models



*p < 0.05, ***p < 0.001. Data are presented as mean + SEM

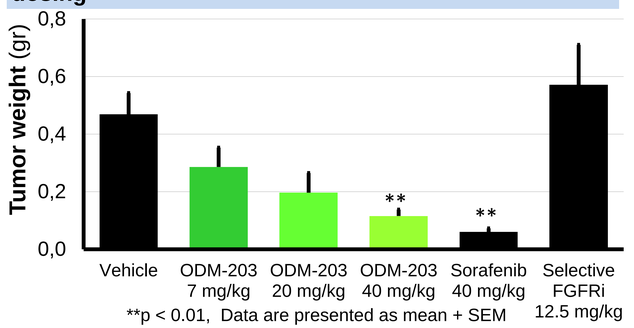
5. ODM-203 inhibits tumor pFGFR in vivo



***p < 0.01, Data are presented as mean + SEM

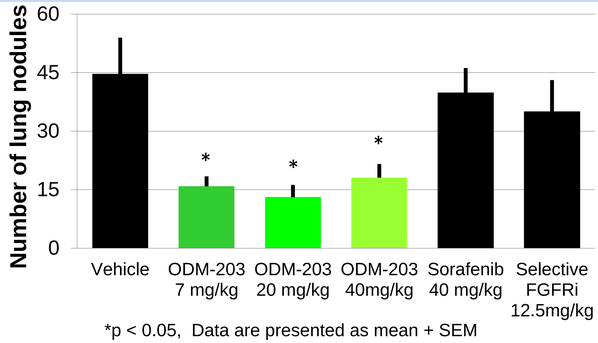
6 ODM-203 shows strong anti-tumor activity in VEGFR dependent orthotopic xenograft model (Renca)

Figure 6A. Primary tumor growth after 21 days of dosing



**p < 0.01, Data are presented as mean + SEM

Figure 6B. Effects on lung metastasis 21 days after dosing



*p < 0.05, Data are presented as mean + SEM

8. ODM-203 is unique as it is equally potent in FGFR and VEGFR dependent models

	Kinase assay	Cell based	In vivo
Drug	FGFR1/VEGFR2	FGFR/Angiogenesis	
ODM-203	1:1	1:4	1:2
Lucitanib	1:5	1:120	ND
Selective FGFRi	200:1	25:1	#

ND = not determined; # = activity only in FGFR-dependent model

ODM-203 has shown no off-target toxic effects in up to 28 days of repeated dosing to rats and dogs.

Conclusions

- ODM-203 is a unique selective inhibitor with equal potency for both FGFR and VEGFR kinase families
- Promising anti-tumor activity in both FGFR-dependent and angiogenic tumor models
- Evidence of reduced metastasis formation
- Only FGFR and VEGFR inhibition related findings observed in pre-clinical toxicity studies
- A clinical trial with ODM-203 is ongoing in patients with solid tumors (NCT 02264418)

Poster presented at AACR 2015
Philadelphia, USA 18- 22 April 2015

These studies were sponsored by Orion Corporation Orion Pharma