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Original Research

Targeting FGFR2 with alofanib (RPT835) shows potent activity in tumour models

Ilya Tsimafeyeu [a,*], John Ludes-Meyers [b], Evgenia Stepanova [c], Frits Daeyaert [d], Dmitry Kochenkov [c], Jean-Baptiste Joose [e], Eliso Solomko [c], Koen Van Akene [e], Nina Peretolchina [c], Wei Yin [b], Oxana Ryabaya [c], Mikhail Byakhov [f], Sergei Tjulandin [c]

[a] *Ruspharmtech LLC, Saint Petersburg, Russia*
[b] *Altogen Labs, Austin, TX, USA*
[c] *N.N. Blokhin Russian Cancer Research Center, Moscow, Russia*
[d] *FD Computing, Beerse, Belgium*
[e] *EcoSynth, Oostende, Belgium*
[f] *Moscow Clinical and Research Center, Moscow, Russia*

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Abstract Alofanib (RPT835) is a novel selective allosteric inhibitor of fibroblast growth factor receptor 2 (FGFR2). We showed previously that alofanib could bind to the extracellular domain of FGFR2 and has an inhibitory effect on FGF2-induced phoshphorylation of FRS2α. In the present study, we further showed that alofanib inhibited phosphorylation of FRS2α with the half maximal inhibitory concentration (IC50) values of 7 and 9 nmol/l in cancer cells expressing different FGFR2 isoforms. In a panel of four cell lines representing several tumour types (triple-negative breast cancer, melanoma, and ovarian cancer), alofanib inhibited FGF-mediated proliferation with 50% growth inhibition (GI50) values of 16–370 nmol/l. Alofanib dose dependently inhibited the proliferation and migration of human and mouse endothelial cells (GI50 11–58 nmol/l) compared with brivanib and bevacizumab. Treatment with alofanib ablated experimental FGF-induced angiogenesis *in vivo*. In a FGFR-driven human tumour xenograft model, oral administration of alofanib was well tolerated and resulted in potent antitumour activity. Importantly, alofanib was effective in FGFR2-expressing models. These results show that alofanib is a potent FGFR2 inhibitor and provide strong rationale for its evaluation in patients with FGFR2-driven cancers.

* *Corresponding author:* Ruspharmtech LLC, Griboyedov Canal Nab. 130 Liter A, Off. 202, Saint Petersburg, 190121, Russia. Tel.: +7 9265646581.
E-mail address: tsimafeyeu@ruspharm.com (I. Tsimafeyeu).

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1. Introduction

Fibroblast growth factors (FGFs) and fibroblast growth factor receptor 2 (FGFR2) regulate cellular proliferation, survival, migration and differentiation. Deregulation of FGF/FGFR2 signalling in cancer is now well understood [2,12,22,28]. The mechanisms of dysregulation of FGFR2 include activating mutations in the extracellular and kinase domains of the receptor [7,25], gene amplification [14,35], chromosomal translocations [32], altered splicing [31,34] and germline single nucleotide polymorphisms [19]. Finally, some studies showed that FGF(R) mediates resistance to vascular endothelial growth factor receptor (VEGFR) targeting by reactivating tumour angiogenesis [9,23,26].

On the basis of the evidence for FGFR dysregulation in tumours, several companies have discovered small-molecule tyrosine kinase inhibitors targeting the ATP-binding site of the intracellular tyrosine kinase domain of FGFRs or monoclonal antibodies blocking the active site of the extracellular domain of FGFRs [1,8,10,27,29,36].

Alofanib (RPT835) is a novel small-molecule selective allosteric inhibitor binding to the extracellular domain of FGFR2 (Fig. 1A). Alofanib had a dramatic inhibitory effect with IC50 <10 nM on FGF2-induced phosphorylation of FRS2α in KATO III cells [21]. However, alofanib had no direct effect on FGF2-dependent FGFR1 and FGFR3 phosphorylation levels in either cell lines. In a non-radioactive high-throughput binding assay and in a binding assay with anti-FGF2 antibody coated wells, there were no effects of alofanib on FGF2-FGFR2 binding.

In this report, we describe the preclinical profile of alofanib.

2. Materials and methods

2.1. Alofanib

3-(*N*-(4-methyl-2-nitro-5-(pyridin-3-yl)phenyl)sulfamoyl)benzoic acid (RPT835, CAS Registry Number 1612888-66-0; Ruspharmtech LLC, Fig. 1B) was synthesized by EcoSynth (Oostende, Belgium) according to the processes described in the International Patent Application Publication Number PCT/EA2014/000013. The free base of alofanib (molecular weight = 413.4 g/mol) was used in all the preclinical studies.

2.2. Cell lines

The following cell lines were purchased: SKOV3, HS578T, NCI-H226, SVEC-4-10, HUVEC, and hFOB from the American Type Culture Collection; SUM 52PE from Asterand; and Mel Kor from the N.N. Blokhin Russian Cancer Research Center (Russian Patent Number 2287578).

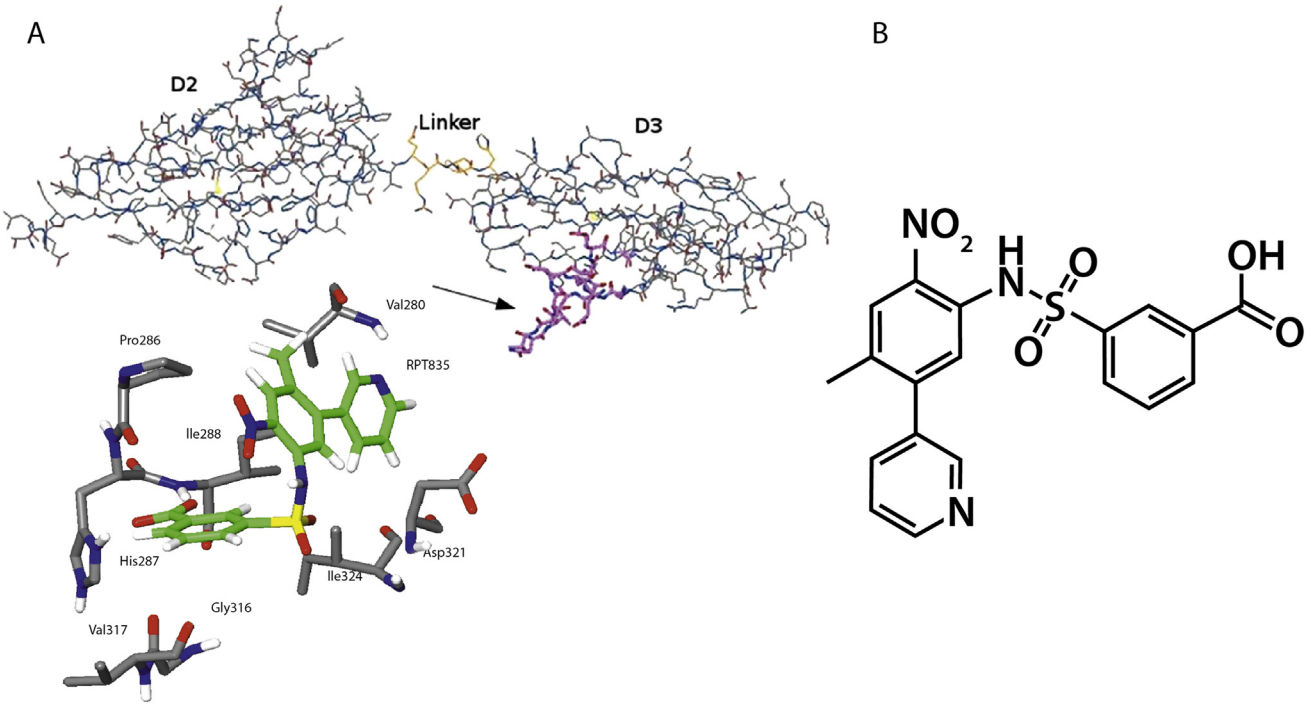

Fig. 1. A, Location of the proposed binding site of alofanib (RPT835) indicated by the arrow. The carbon atoms of the linker between D2 and D3 of fibroblast growth factor receptor 2 are colored orange. The carbon atoms of amino acids 313–325 are colored magenta. B, Chemical structure of alofanib.(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)