Highly cytotoxic FGF2-conjugates in targeted therapy for FGFR-expressing cancers

**Beneficiary:** University of Wrocław
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Akronim: FGF2CON
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Project objectives

Production and biophysical characterization of FGF2 and FGF2-conjugates suitable for targeted therapy of FGFR-related cancers

- Design and production of recombinant FGF2 proteins
- Cytotoxic FGF2-conjugates preparation
- Biophysical characterization of FGF2-conjugates

Testing and characterization of FGF2 and FGF2-conjugates suitable for targeted therapy of FGFR-related cancers in cellular model – cell line in vitro study

- Analysis of biological activities of FGF2 constructs
- Cytotoxicity studies of FGF2 variants and their conjugates
- Examination of the effect of simultaneous use of FGF2-conjugates and specific FGFR tyrosine kinase inhibitors

In vivo studies in mouse xenograft models for human cancer and validation of proposed therapy

- Maximum tolerated dose estimation studies of cytotoxic drugs and cytotoxic FGF2-conjugates
- Biodistribution and tumor accumulation of fluorescently labeled FGF2
- The normal tissue toxicity of FGF2-conjugates
- Antitumor activity of FGF2-conjugates alone or in combination with FGFR tyrosine kinase inhibitor
Methods and approach

• Protein production and purification (E. coli expression system, heparin affinity purification)
• FGF2-cytotoxic drug conjugation (monomethyl auristatin E, doxorubicine or epirubicine)
• Biophysical studies (MS MALDI TOF or ESI MS/MS, circular dichroism and fluorescence techniques)
• Protein-protein interaction studies (SPR- BIAcore3000, fluorescence polarization methods)
• Receptor binding assay (competitive binding assay, label-free real time assay)
• Microscopy (fluorescence and confocal)
• Activation of signaling pathway (Western blotting)
• Proliferation and cytotoxic studies ([³H]thymidine incorporation, colorimetric (MTT) or fluorescent (AlamarBlue) assays)
• In vivo studies (mouse model, biodistribution and tumor accumulation, in vivo fluorescent imaging, subcutaneous xenografts and metastasis model; tumor analysis and histological examination of major organs)