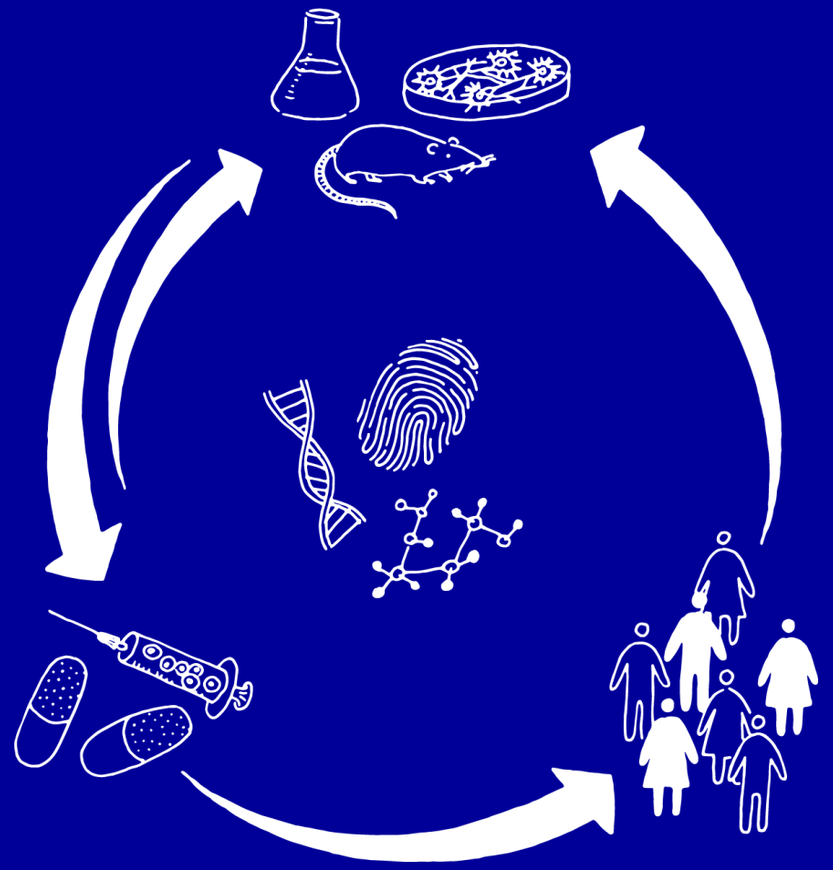
evotec

#RESEARCHNEVERSTOPS

Translation to clinical success by using state-of-the-art bio-markers assays & multi-omics – the story of EVT801

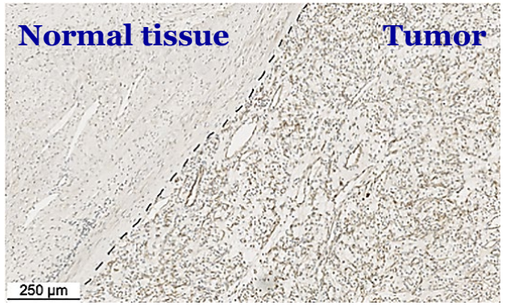


EVT801 - a selective VEGFR-3 inhibitor

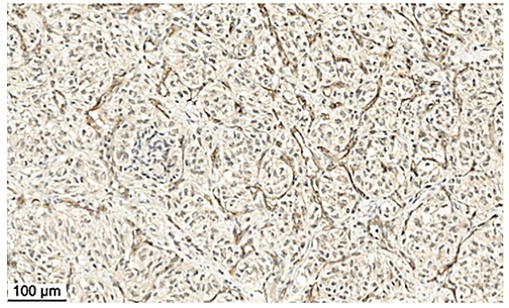
Targeting tumor angiogenesis in comparison to angiokinase inhibitors

VEGFR-3 is expressed on tumor blood vessels

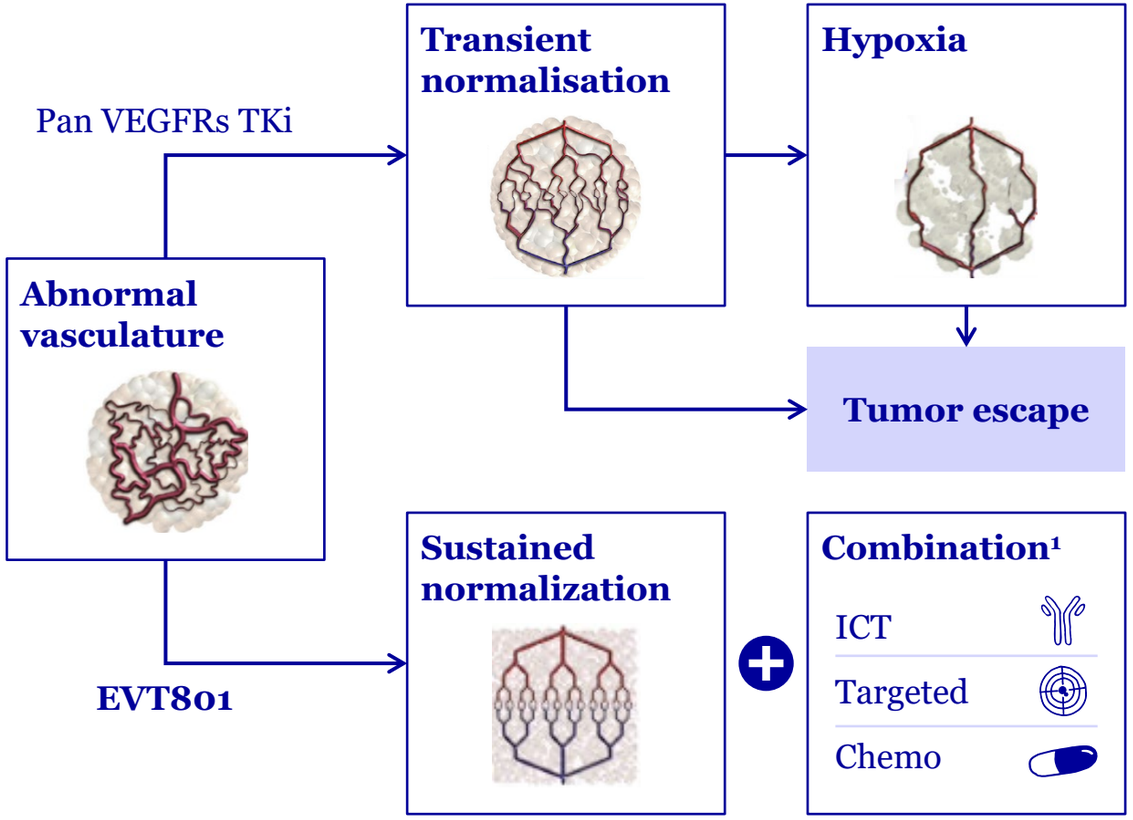
Human primary kidney cancer
VEGFR3 labelling



IHC image of VEGFR-3 expression in bone metastasis after treatment with sunitinib



Concept to target tumor angiogenesis for patients with immune-excluded tumors



EVT801 is a selective VEGFR-3 inhibitor

Recombinant enzymes

Recombinant enzymes	EVT801 (IC_{50})
VEGFR-1	396 nM
VEGFR-2	125 nM
VEGFR-3	**11 nM**

$VEGFR3^{pos}$ Endothelial cells

hLMVEC pErk1/2	EVT801 (IC_{50})
VEGF-C (VEGFR-2/-R3 ligand)	**13 nM**

hLMVEC proliferation	EVT801 (IC_{50})
VEGF-C (VEGFR-2/-R3 ligand)	**11 nM**
VEGF-D (VEGFR-3/-R3 ligand)	**16 nM**

Scheme adapted from Bergers & Hanahan, *Nat Rev Cancer*. 2010 and R. Jain Cancer cell, 2014
1 As function of indication

VEGFR3 expression on tumors and TME defines 3 distinct groups[1]

Selection of indications to maximize the probability of EVT801 response

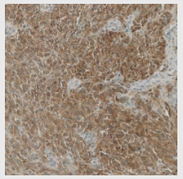
1 Orphan diseases

High Tumor VEGFR3^{pos}

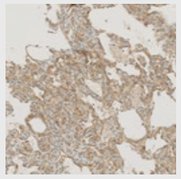
Cancers that express VEGFR-3 on the tumour

VEGFR-3 expression

Kaposi sarcoma



(Lymph) angiosarcoma

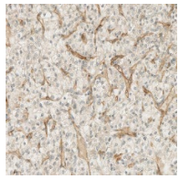


2

High TME VEGFR3^{pos}

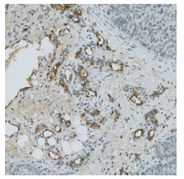
Cancers that highly express VEGFR-3 in the stromal environment

Clear-cell renal-cell carcinoma[2]

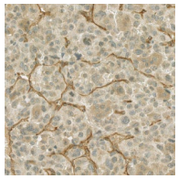


Hepato-carcinoma

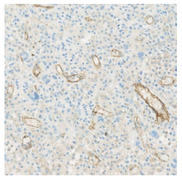
HGS ovarian cancers



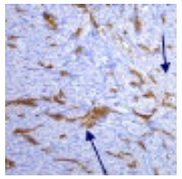
Osteosarcoma



Synovial, Ewing pleio-morphic sarcomas



Some Head & Neck cancers



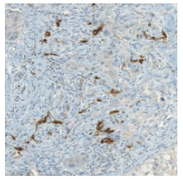
3

Intermediate TME VEGFR3^{pos}

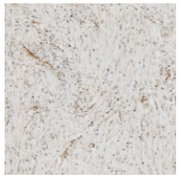
Cancers that highly express VEGFR-3 in the stromal environment

VEGFR-3 expression

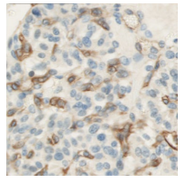
Lung cancer



Colo-rectal cancer



Pancreatic cancers



TECENTRIQ® (Atezolizumab) is FDA-approved in NSCLC, SCLC, HCC, melanoma, UC, TNBC, ASPS

Tumor immune phenotype[3]

Indication		Immune inflamed	Immune excluded	Immune desert
Incidence	CRC	12%	70%	25%
	NSCLC	31%	44%	25%
	mUC	26%	47%	17%
	TNBC	36%	47%	17%

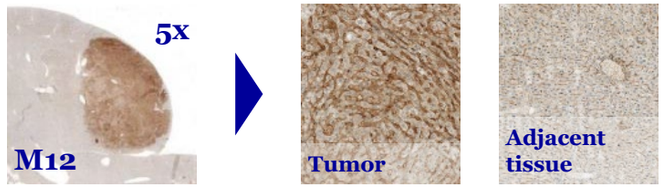
1 based on VEGFR3 expression and localization; Melanoma, triple negative breast, gastric, urothelial cancers were not investigated; head & neck cancers requires more investigations
2 Same VEGFR3 expression was observed on metastatic biopsies from patients with ccRCC treated with anti-angiogenic drugs
3 Data from Genentech
TME tumor micro environment; HGS: high grade serous; CRC: colorectal cancer; SCLC: Small cell lung cancer; NSCLC: Non small cell lung cancer; mUC: metastatic urothelial cancer; TNBC: Triple negative lung cancer; ASPS: Alveolar soft part sarcoma

EVT801 inhibits tumor growth in models with high VEGFR-3^{pos} in TME

Hepatocarcinoma and pancreatic tumor mouse models

DEN-induced liver tumor mouse model

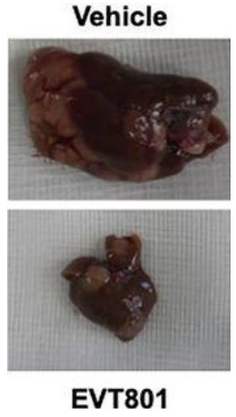
Model characterization at month 12



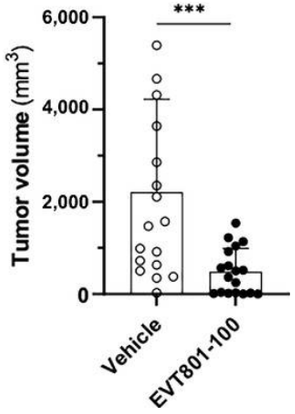
VEGFR3 is highly expressed in sinusoids inside tumor: like HBV-induced HCC

DEN-induced liver tumor mouse model data after 2 months of daily treatment with vehicle or 100 mg/kg EVT801

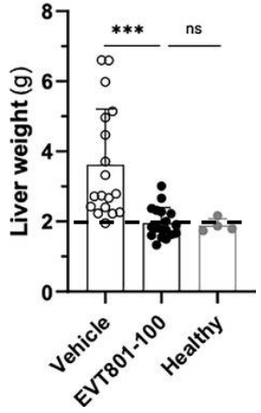
Representative whole liver



Tumor volume

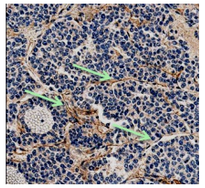


Liver weight



Transgenic Rip1-Tag2 pancreatic tumor mouse model

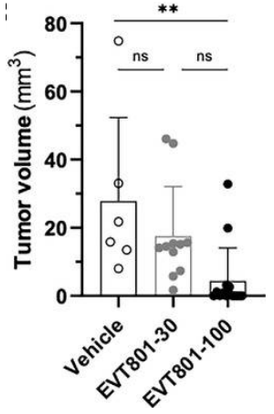
Model characterization



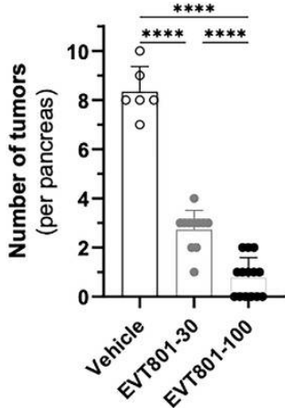
VEGFR3 is highly expressed on endothelial cells inside tumor

Orthotopic Rip1-Tag2 tumor volume after 2 weeks of daily treatment with vehicle, 30 mg/kg EVT801 or 100 mg/kg EVT801

Tumor volume

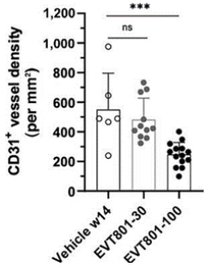


tumors per pancreas



Representative IHC images

Intratumor vascular density

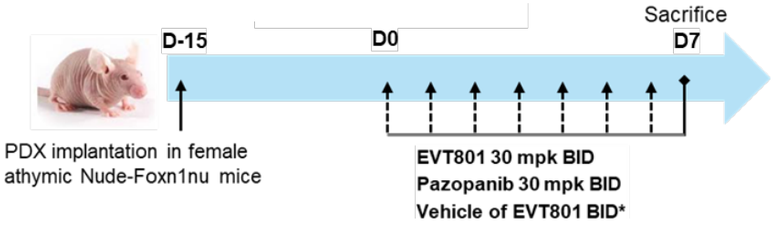


DEN: Diethylnitrosamine
TME: Tumor micro environment
HCC: hepatocarcinioma

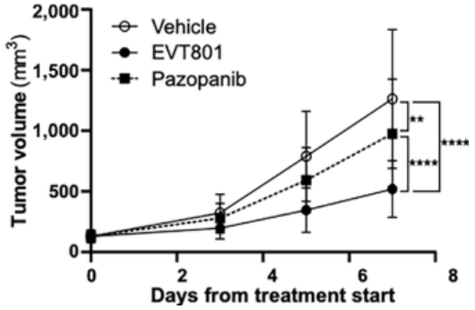
EVT801 is effective in PDX model with intermediate VEGFR-3^{pos} in TME

Inhibits tumor growth, normalizes vasculature and reduces hypoxia

Rhabdomyosarcoma RH-HAM-001 PDX model

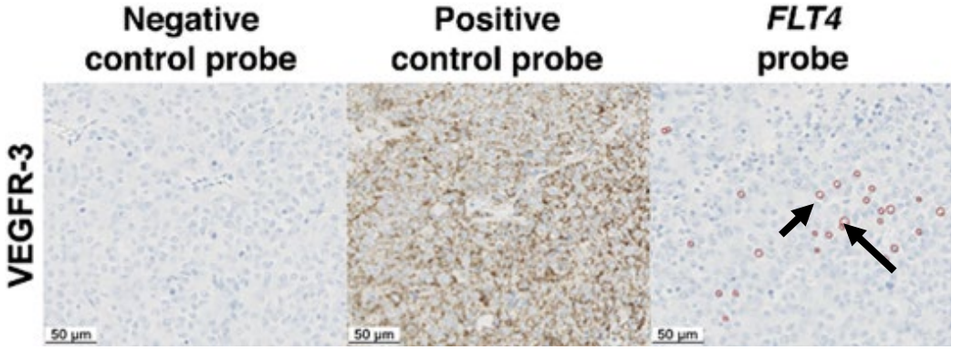


EVT801 more effective than pazopanib in sarcoma PDX model



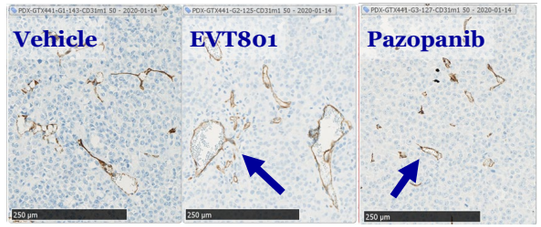
Representative ISH image of hFLT4 mRNA expression in RH-HAM-001 PDX tumors at Day 0

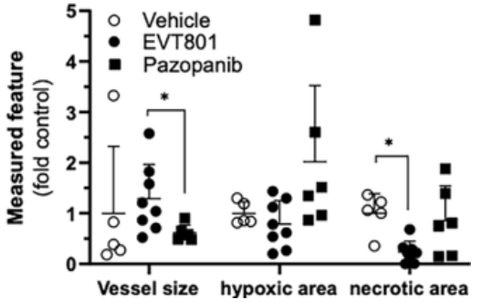
Hs-PPIB is used as positive control to validate FLT4 mRNA staining



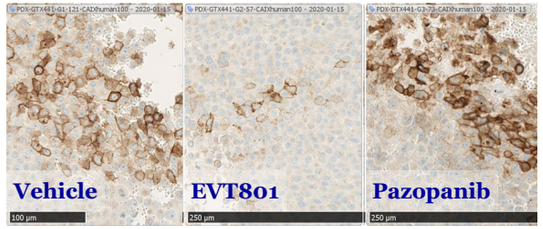
20% of cells in RT-HAM-001 tumors expressed detectable amounts of FLT4 (VEGFR3) mRNA

CD31: a marker for blood vessels





CAIX: a marker of hypoxia



EVT801

- Induces tumor normalization
- Reduces hypoxia & necrosis

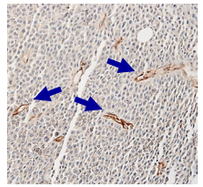
TME: Tumor micro environment
PDX: Patient-derived xenograft

EVT801 enhances ICT efficacy in models with intermediate VEGFR-3^{pos}

Inhibits tumor growth, reduces lung metastasis, and increases CD8^{pos} T-cells infiltration

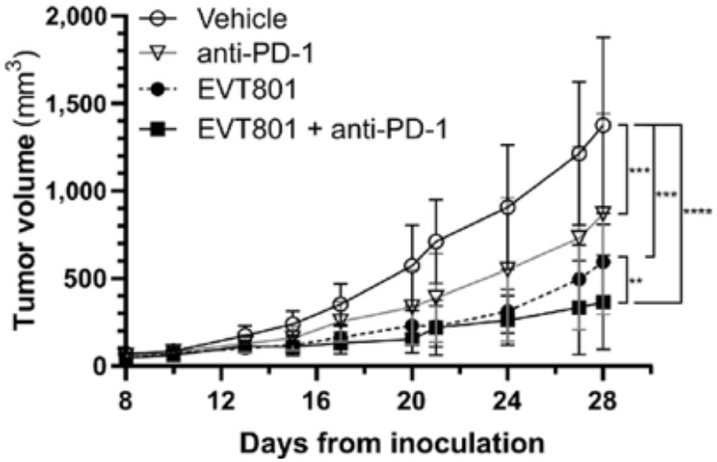
Orthotopic 4T1 tumor in BALB/c mice

Model characterization at Day 7

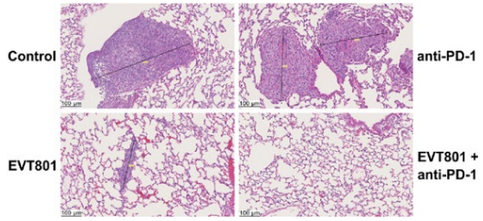


VEGFR3 is weakly expressed in endothelial cells inside tumor

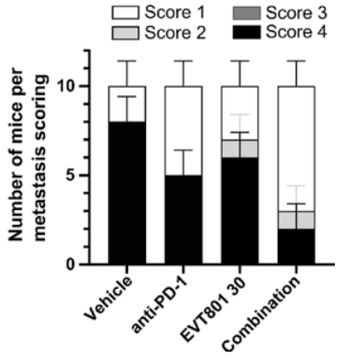
Tumor volume progression in presence of vehicle, anti-PD-1, EVT801 (30 mg/kg/BID) or a combination



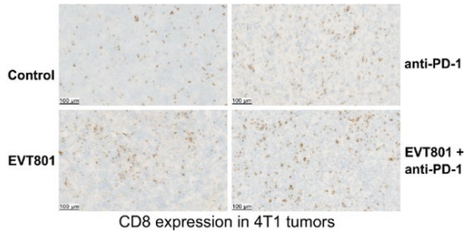
Quantification of lung metastasis at endpoint



Representative HE staining of 4T1 lung metastasis

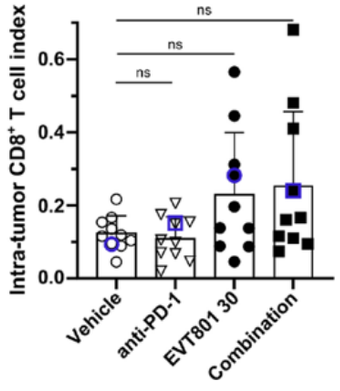


Frequency of CD8^{pos} T cells in 4T1 tumors at endpoint



Representative IHC images of CD8^{pos} T-cells via CD8 expression in 4T1 tumors at endpoint

The highlighted datapoint in blue refers to the samples that were used for IHC imaging

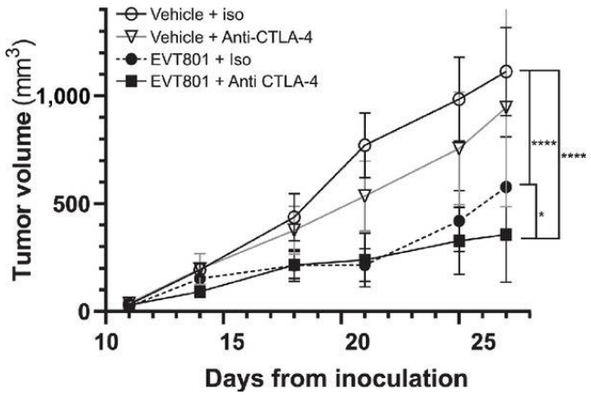


TME: Tumor micro environment
BiD: Bis in die (twice a day)
HE: hematoxylin-eosin
IHC: immunohistochemistry

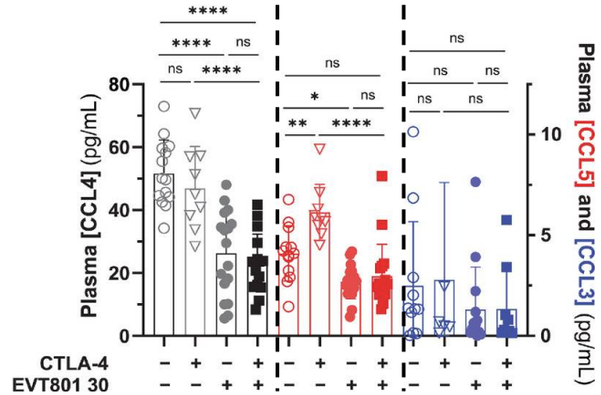
EVT801 reduces circulation of chemokines & MDSCs in the blood

EVT801 increases anti-CTLA-4 mAb efficacy on orthotopic 4T1 tumor model (weak VEGFR-3^{pos} in TME)

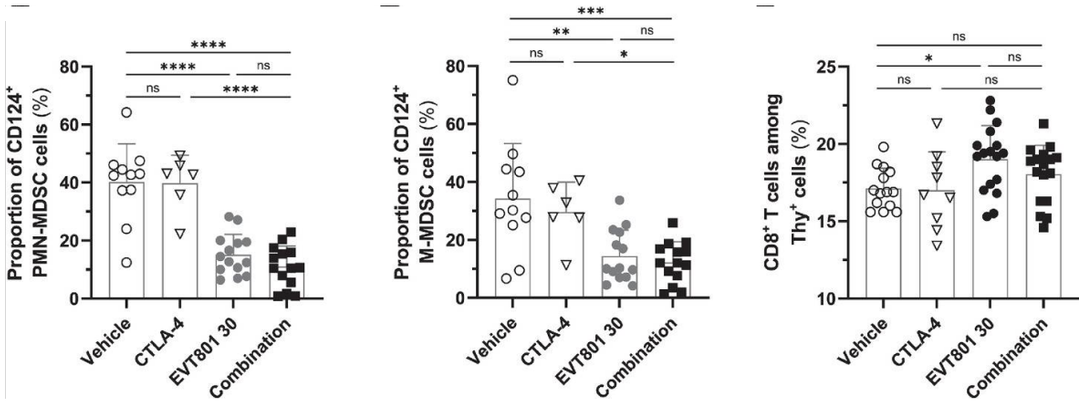
Tumor volume progression in presence of vehicle, anti-CTLA-4, EVT801 (30 mg/kg/ BID) or a combination



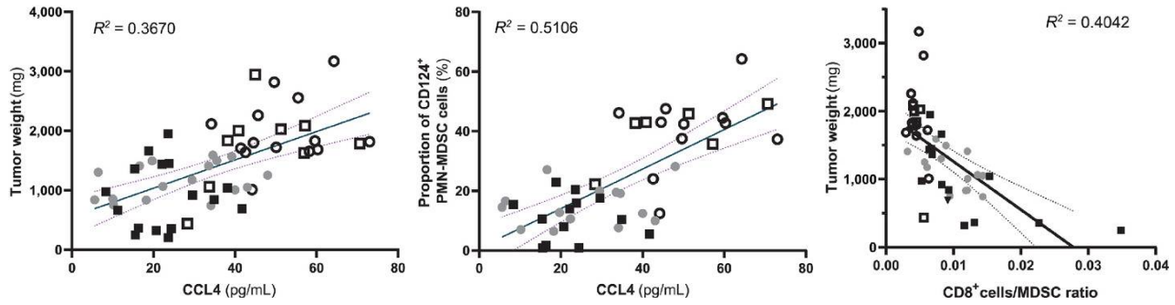
Plasma concentrations of CCL3/MIP-1α, CCL4/MIP-1β & CCL5/Rantes at endpoint



Blood concentration of CD124^{pos} PMN-MDSCs, CD124^{pos}, M-MDSCs & CD8^{pos} T-cells at endpoint



Correlation between circulating CCL4/tumor weight & PMN-MDSC/tumor weight; Inverse correlation between CD8^{pos} T-cell/PMN-MDSC ratio & tumor weight



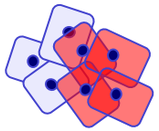
MDSC: myeloid-derived suppressor cells
TME: Tumor micro environment
BiD: Bis in die (twice a day)
IHC: immunohistochemistry

EVT801 induces tumor blood vessels normalization

Schematic overview based on pre-clinical data

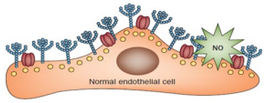
EVT801 activity on tumor microenvironment

Normalization of tumor (lymph)-angiogenesis



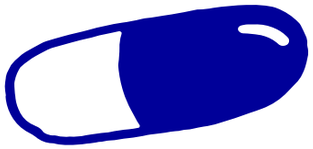
Hypoxia

CD8^{pos} T-cell infiltration



Endothelial cell immunotolerance

Tumour associated macrophages

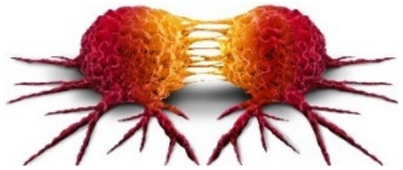


EVT801 *(SAR131675)*[1]

Cytokines involved in MDSC frequency

Myeloid-derived suppressor cells

Tumor metastasis



Multiple cooperative modes of action

EVT801 MoA hypothesis: by destructing VEGFR3^{pos} tumor blood vessels, EVT801 would induce tumor blood vessels normalization, reduce hypoxia, and improve CD8 T-cells infiltration

Data from Tacconi & al. with SAR131675

1 SAR131675, a close analogue of EVT801

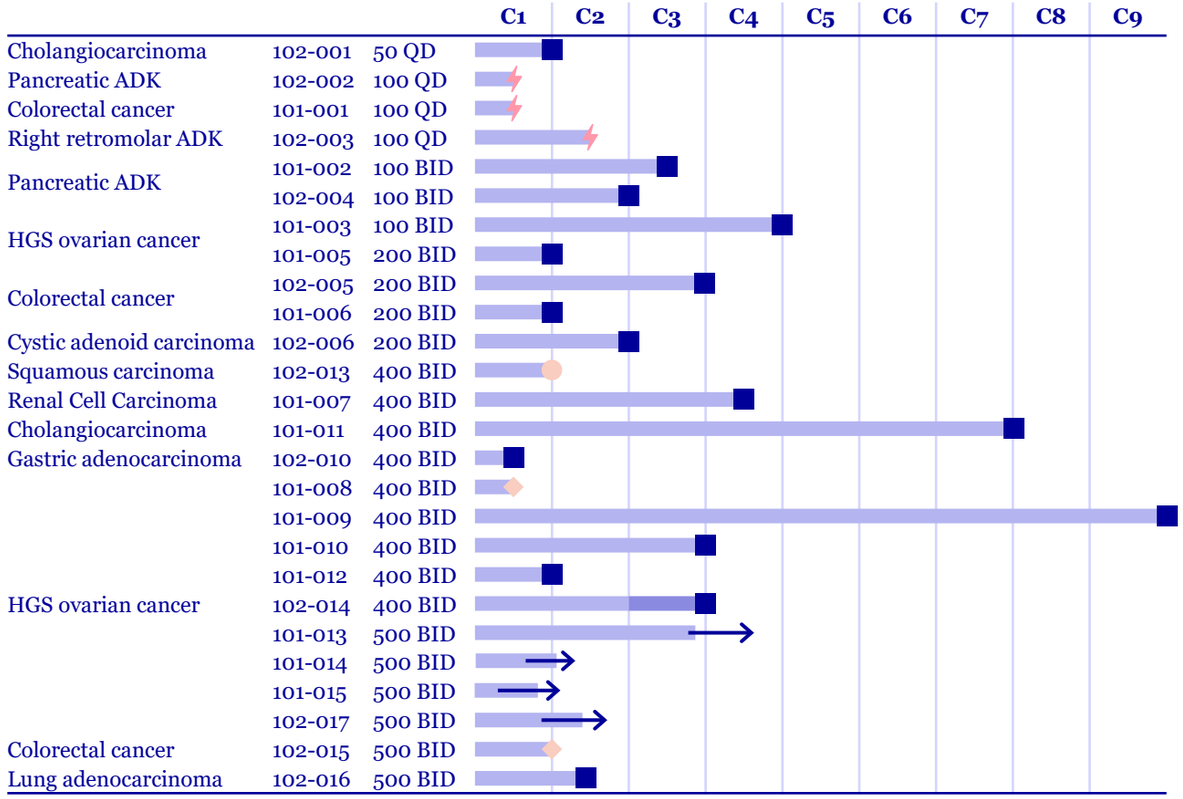
C. Tacconi & al., Cancer Res; Activation of the VEGFC/VEGFR3 pathway induces tumor immune escape in colorectal cancer Aug. 201A.Alam & al. BBRC, 324 (2004) 909–915
A.Alam & al. BBRC, 324 (2004) 909–915; Heterodimerization with vascular endothelial growth factor receptor-2 (VEGFR-2) is necessary for VEGFR-3 activity
A.Alam & Al.SAR131675, a Potent and Selective VEGFR-3–TK Inhibitor with Antilymphangiogenic, Antitumoral, and Antimetastatic Activities Mol Cancer Ther. 2012; 11(8):1637-49

Patient overview

26 patients with various types of diseases were treated with EVT801 at different doses

Currently 32 patients included in stage 1

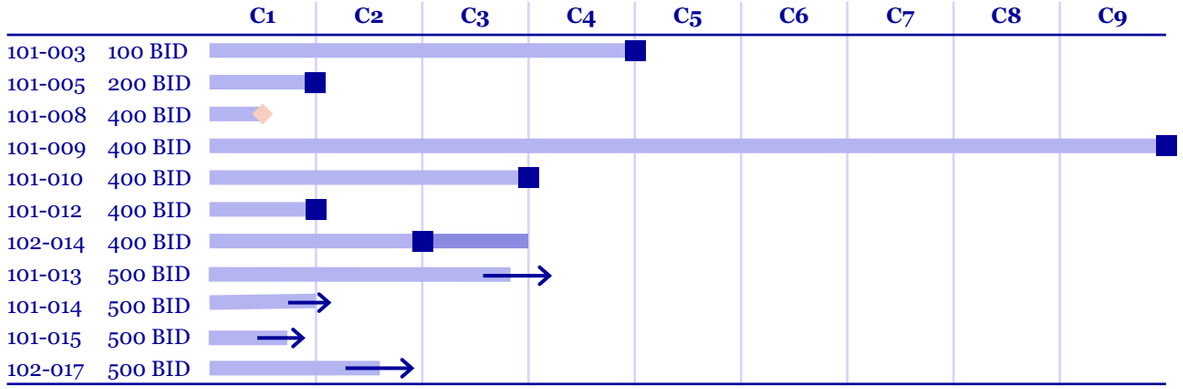
- 6 Screening failure
- 26 patients treated within 6 cohorts at different doses (50mg QD to 500 mg BID)



Human active dose prediction based on predicted human clearance of 2.5 mL/min/kg: **375 mg BID***. A conservative estimate of human clearance (4 mL/min/kg) would drive to a dose of **600 mg BID***

Focus on patients with Ovarian Cancer

Status on 29th of February 2024



Stop for IMP incompliance | IMP taken after Progressive disease | Stable disease (SD) | → *Ongoing* Treatment | Progressive disease (PD) | Dose Limiting Toxicity | Stop for adverse event

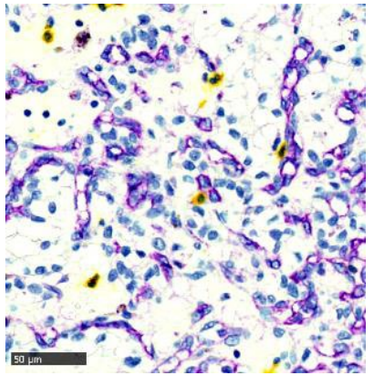
QD = *Quaque Die* (once a day); BiD: Bis in Die (twice a day); IMP: Investigational Medicinal Product

EVT801 – Exploratory Biomarkers in FIH trials

From TE/PD markers to safety to stratification

Patient stratification based on VEGFR-3 expression in archival tissues and/or biopsies

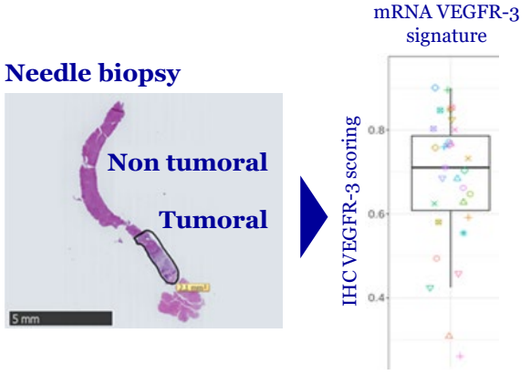
- VEGFR-3 signature by IHC:
  - VEGFR-3/CA9/CD8/CD31/PD-L1



Example VEGFR-3/Immune infiltration (CD8) in renal cell carcinoma

VEGFR-3 & response to immune checkpoint therapies mRNA signature

- VEGFR-3 mRNA signature
- PD-1 response mRNA signature on archival tissues and/or biopsies



Circulating drug related biomarkers

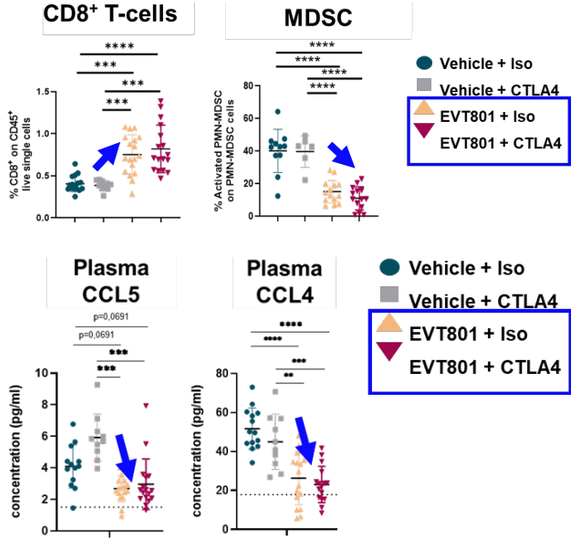
Bulk RNA sequencing on blood cells at C1D1 vs C2D1 (Paxgene tube)

Safety biomarkers to control hypertension

Blood pressure measurement to control that EVT801 does not induce hypertension (as demonstrated in preclinical model)

Circulating endpoint biomarkers

- **Immunomonitoring** based on CD8⁺ T-cells/ MDSC ratio at C1D1 vs C2D1
- **Proteins signature** based on chemokines involved in inflammation & angiogenesis at C1D1 vs C2D1



Resting samples will include

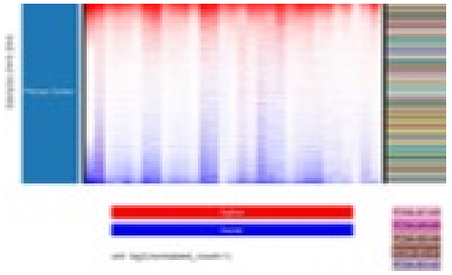
- Frozen whole blood & plasma
- Frozen PBMCs
- FFPE biopsies

Patient characterization from FFPE archival tissues

Established link between clusters of genes and tumor micro-environment

Data mining

- Describe predictive signature to treatment
- Target co-regulated genes
- Validation of the signature using Fluidigm
- Transfer to Nanostring technology during clinical trials

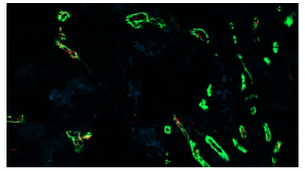


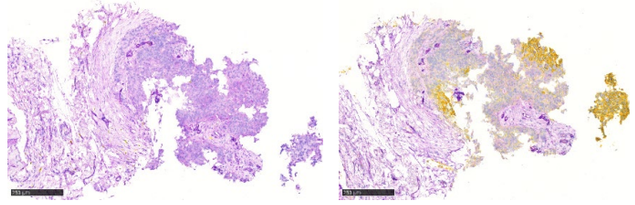
Identification of gene signature from the literature of from publicly available dataset

FFPE Archival tissues to provide mRNA and protein expression data

Histology labeling

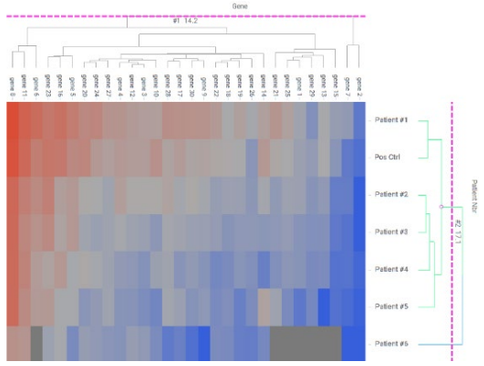
- tumor architecture to identifier the regions to collect RNA
- Labelling of tumor micro-environment:
  - Immune infiltration
  - Vascularization



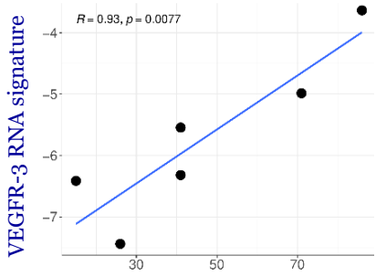


RNA signature from limited quantities of material including needle biopsies

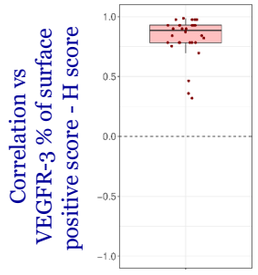
- RNA signature developed by Evotec bio-informatic department



Correlation of RNA signature and VEGFR-3 expression (p=0.0077)

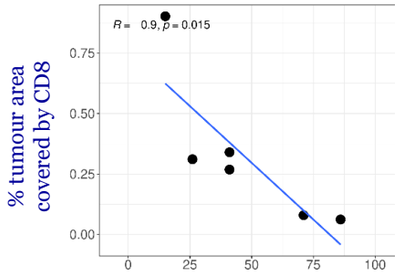


VEGFR-3 IHC % of surface positive score - H score

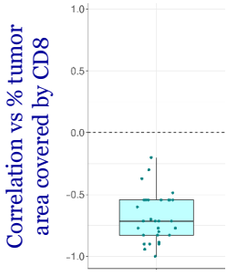


VEGFR-3 RNA signature

Establishment of an anti-correlation between VEGFR-3 expression and immune infiltration



VEGFR-3 % of surface positive score - H score



VEGFR-3 RNA signature

HGS-OC patients and EVT801

First clinical data suggests a beneficial effect

EVT801:

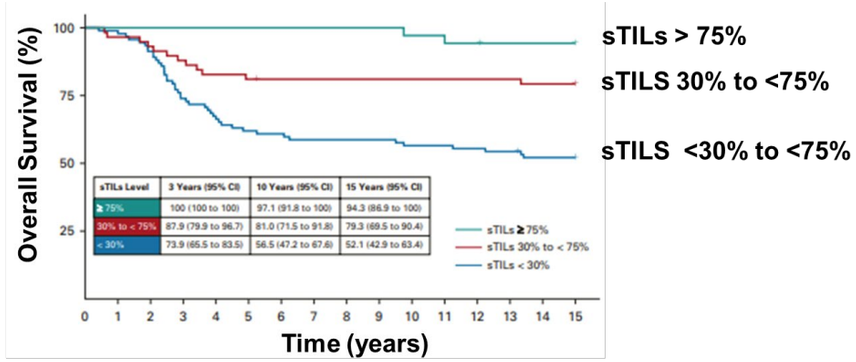
- EVT801 induces synergistic activity with immune checkpoint therapies in the preclinical studies
- **First clinical data shows that EVT801 could be beneficial for high grade serous ovarian cancer** (HGS-OC)

BUT…

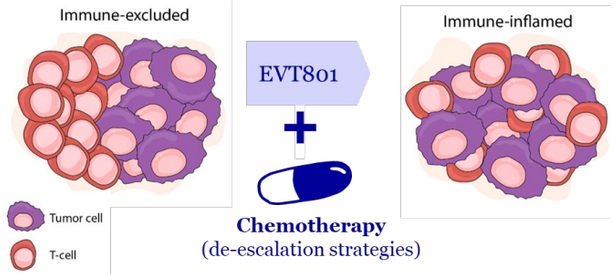
- Immune checkpoint therapies are not recommended in HGS-OC
- Platinum/taxane chemotherapies (+/- Bevacizumab) are SoC (+PARP inh. for HGS-OC patients with mutant BRCA1/2 or HR defective)

However, increased stromal TILs, as seen with EVT801, have been associated with a strong prognostic factor for overall survival in TNBC & HGS-OC

- In patients treated with and without (neo)-adjuvant chemotherapy
- Survival is strongly correlated with CD8⁺ TILs on immune inflamed phenotype than in immune exclude



Kaplan-Meier curves for OS according to sTILS categories in patients with TNBC T2/3 tumors



Understanding factors driving T-cell infiltration will be key to unravel the clinical outcome heterogeneity.

Identifying new drugs that drive T-cell infiltration into the tumor would be pivotal for:

- Investigating (neo)adjuvant chemotherapy de-escalation strategies
- Identifying select subsets of OC patients who may benefit from new therapies

HGS-OC: High grade serous ovarian cancer, TNBC: Triple negative breast cancer TILs: tumor infiltrating lymphocytes, SoC = Standard of Care

Prognostic Value of Stromal Tumor-Infiltrating Lymphocytes in Young, Node-Negative, Triple-Negative Breast Cancer Patients Who Did Not Receive (neo)Adjuvant Systemic Therapy Vincent M.T. de Jong & al. J Clin Oncol 40:2361-2374. © 2022
Prognostic and Predictive Role of Tumor-Infiltrating Lymphocytes (TILs) in Ovarian Cancer Daniele Fanale & al.; Cancers 2022, 14, 4344.
Prognostic Value of Tumor-Infiltrating Lymphocytes in Triple-Negative Breast Cancers From Two Phase III Randomized Adjuvant Breast Cancer Trials: ECOG 2197 and ECOG 1199 ; Sylvia Adams & al. JOURNAL OF CLINICAL ONCOLOGY, 32/27, 2014

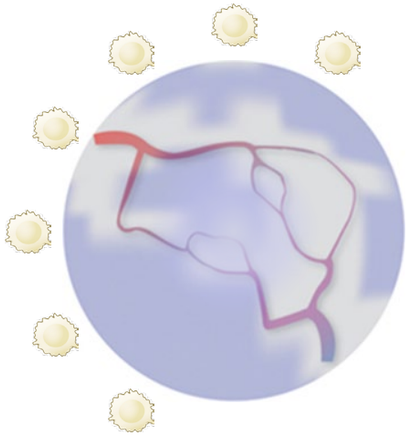
Overall EVT801 combination strategy for clinical trial phase 2

Hypothesis coming from high grade serous ovarian cancer patients' analysis

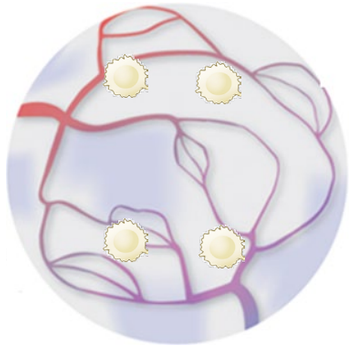
Working hypothesis for EVT801 to enhance the benefit of current SoC (add-on) in multiple cancer types:

Patient characterization

- **IHC**
  - $CD8^{neg}$ or only at the edge
  - $VEGFR\text{-}3^{pos}$
  - $CA9^{pos}$
- **mRNA signature**
  - VEGFR3 high
  - PD1 response
  - Hypoxia?



EVT801



EVT801

EVT801 in combo

- Tumor vessel normalization
- Less hypoxic tumor
- Enhanced immune cells infiltration

Potential Combination[1]

Immune check-point therapy

or

Targeted therapy

or

Chemotherapy

I.e. TNBC & HGS-OC

Hypoxia Normoxia Vascularisation $CD8^{pos}$ T-cells

1 As function of indication

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Evotec Clinical Operations team

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Philippe Rochaix
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Biological Resources Centre (Dr Anne Gomes-Mascard)

The patients & their families

#RESEARCHNEVERSTOPS

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EVT801, started enrollment for its first-in-human study

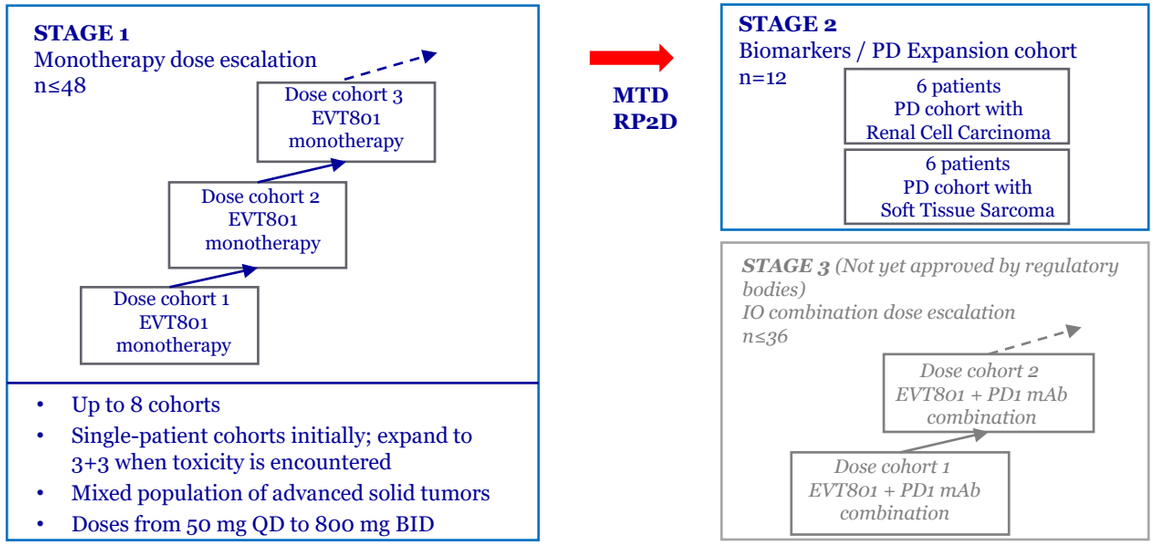
EVT801 was licensed to Kazia therapeutics in April 2021

KAZIA THERAPEUTICS

A Phase 1, First-in-Human, Open-Label Study to Assess the Safety, Tolerability and Pharmacokinetics of EVT801 in Patients with Advanced Solid Tumours

Sponsor : Kazia Therapeutics Ltd
Product : EVT801
EudraCT Number : 2021-002483-47

Clinical sites (France only) :
IUCT-Oncopole, Toulouse - PI : Dr Gomez-Roca
Centre Léon Bérard, Lyon – PI : Dr Philippe Cassier



- Up to 8 cohorts
- Single-patient cohorts initially; expand to 3+3 when toxicity is encountered
- Mixed population of advanced solid tumors
- Doses from 50 mg QD to 800 mg BID

Stage 2: RCC: renal cell carcinoma; STS: soft tissue sarcoma; High grade serous (HGS) ovarian cancer under consideration

Approvals from regulatory bodies obtained in September 2021

- First-Patient-In in Oct 2021
- 2 clinical sites in France (Toulouse IUCT and Lyon CLB)

INSTITUT UNIVERSITAIRE DU CANCER DE TOULOUSE Oncopole

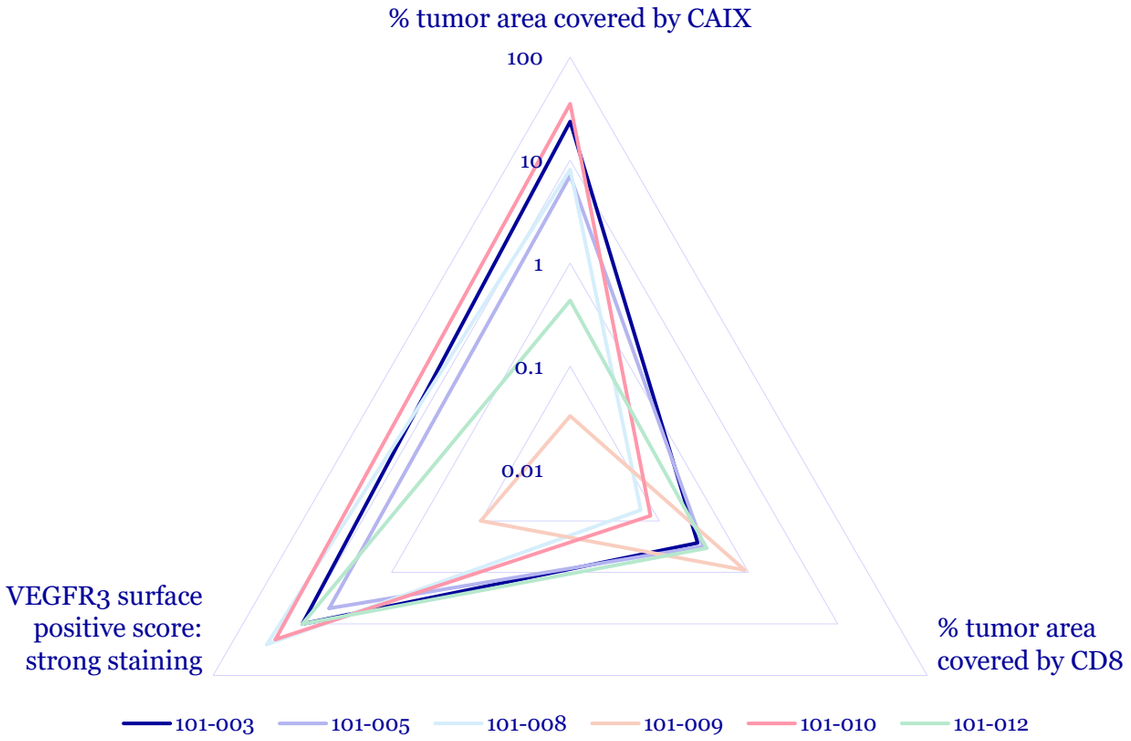
CENTRE DE LUTTE CONTRE LE CANCER LEON BERARD

Primary Objective	• To evaluate the safety and tolerability of EVT801 in subjects with advanced or metastatic solid tumours • To determine the maximum tolerated dose (MTD) and / or a recommended Phase 2 dose (RP2D) of EVT801 when administered daily to subjects with advanced or metastatic solid tumours
Secondary Objectives	• To characterise the pharmacokinetics (PK) of EVT801 following administration in an oral capsule formulation • To identify active metabolites of EVT801 in plasma • To determine preliminary anti-tumour activity of EVT801 via assessment of overall response rate (ORR)
Exploratory Objectives	• To explore the pharmacodynamic effects of EVT801 • To calculate progression-free survival (PFS) and overall survival (OS) for patients treated with EVT801 • **To investigate potential biomarkers of activity of EVT801 by bio-chemical and transcriptomics analysis of blood and tumour samples** • **To correlate PD response and ORR to VEGFR3 expression in tumour samples** • **To explore effects of EVT801 on the tumour immune microenvironment**

MTD = Most Tolerated Dose ; RP2D = Recommmended Phase 2 Dose

Overview of IHC quantifications and scoring for all ovarian cancer patients

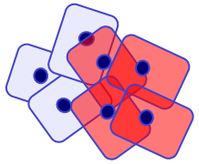
Preliminary results



In ovarian cancers patients, high VEGFR3 expression in vessels seems to be associated with a highest level of hypoxia and to a reduced CD8 infiltration

EVT801 pre-clinical activity on tumour microenvironment

Normalization of tumor (lymph)-angiogenesis ▲



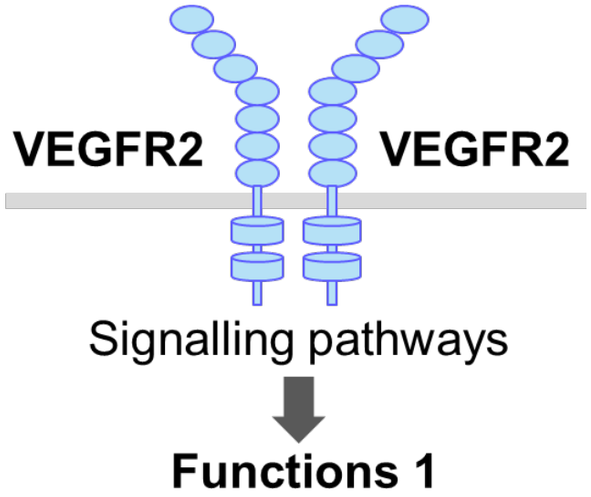
Hypoxia ▼

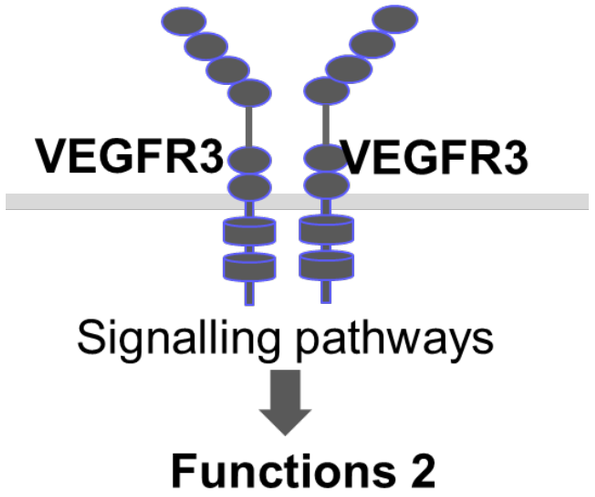
CD8^{pos} T-cell infiltration ▲

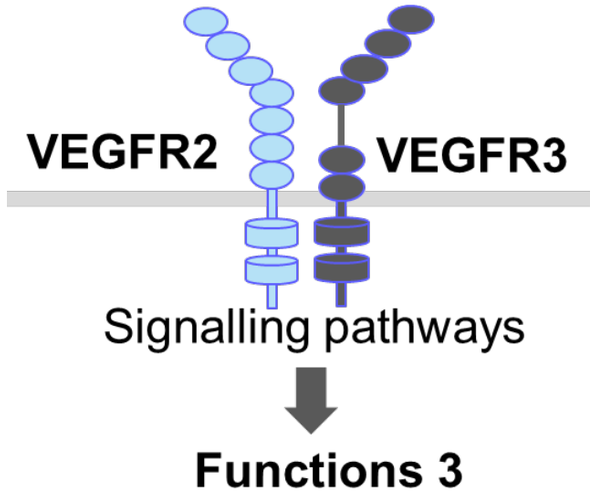
Hypoxic ovarian tumor poorly infiltrated with CD8^{+} T-cells and with high VEGFR3 expression needs to be considered for EVT801 treatment

EVT801 has a unique MoA in comparison to anti-angiogenic mAb

VEGFR-2/-3 heterodimers are pivotal for tumor angiogenesis[1-3]







	Functions 1	Functions 2	Functions 3
Avastin (anti-VEGF-A mAb)	**Inhibition**	–	–
IMC-3C5 (anti-VEGFR3 mAb)	–	**Inhibition**	–
EVT801	–	**Inhibition**	**Inhibition**

1 Heterodimerisation with vascular endothelial growth factor receptor-2 (VEGFR-2) is necessary for VEGFR-3 activity, A.Alam & al. BBRC, 324 (2004) 909–915
2 VEGF receptor 2/-3 heterodimers detected *in situ* by proximity ligation on angiogenic sprouts, I. Nilsson & al. The EMBO Journal (2010) 29, 1377–1388
3 Ligand-induced Vascular Endothelial Growth Factor Receptor-3 Heterodimerization with VEGFR-2 in Primary Lymphatic Endothelial Cells Regulates Tyrosine Phosphorylation Sites, J. Dixelius & al, JBC Vol. 278, No. 42, 40973–40979, 2003

EVT801 activity is correlated with VEGFR3 expression

Basis for patient selection & trial stratification

Tumor Model	Model	VEGFR3 (level) Tumor	VEGFR3 (level) Stroma	T/C ratio[1]
Models with tumor cells expressing VEGFR3				
BNL-R3 HCC	Ectopic Syngeneic	++++	NA	10%
NCIH-1703 Lung cancer	Ectopic Immunodeficient	+++	NA	27%
PDX RT-001-HAM Rhabdomyosarcoma	Ectopic Immunodeficient	+	NA	41%
Models with high VEGFR3 expression in stroma				
RIP1.Tag2 Pancreas	Transgenic Syngeneic	-	+++	- 35%[2]
DEN HCC	Chemo-induced Syngeneic	-	+++	9%[2]
Models with low VEGFR3 expression in stroma (for combination with PD1 mAb)				
4T1 Breast cancer	orthotopic Syngeneic mouse	-	+	48%
CT26 CRC	Ectopic Syngeneic mouse	-	NA	59% (NS)

Active: T/C <42% Intermediate: 42%<T/C<80% T/C>80%

1 T/C ratio expressed here as a percentage is at the last day of the study the (mean tumor volume treated/mean tumor volume control) x 100
2 data obtained with 100 mg/kg QD for which AUC is similar to 30 mg/kg BiD