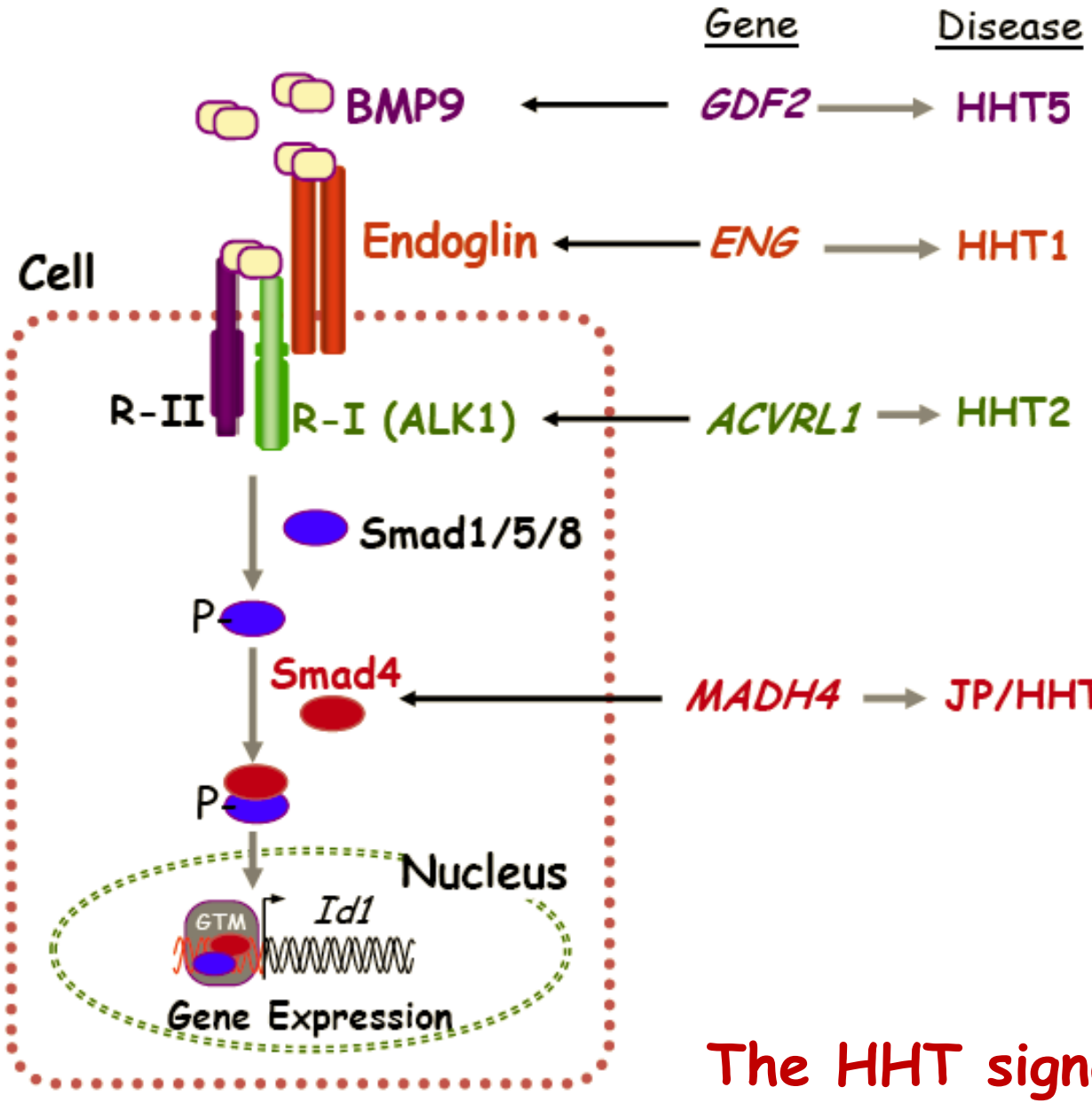
Basic Science

Summary

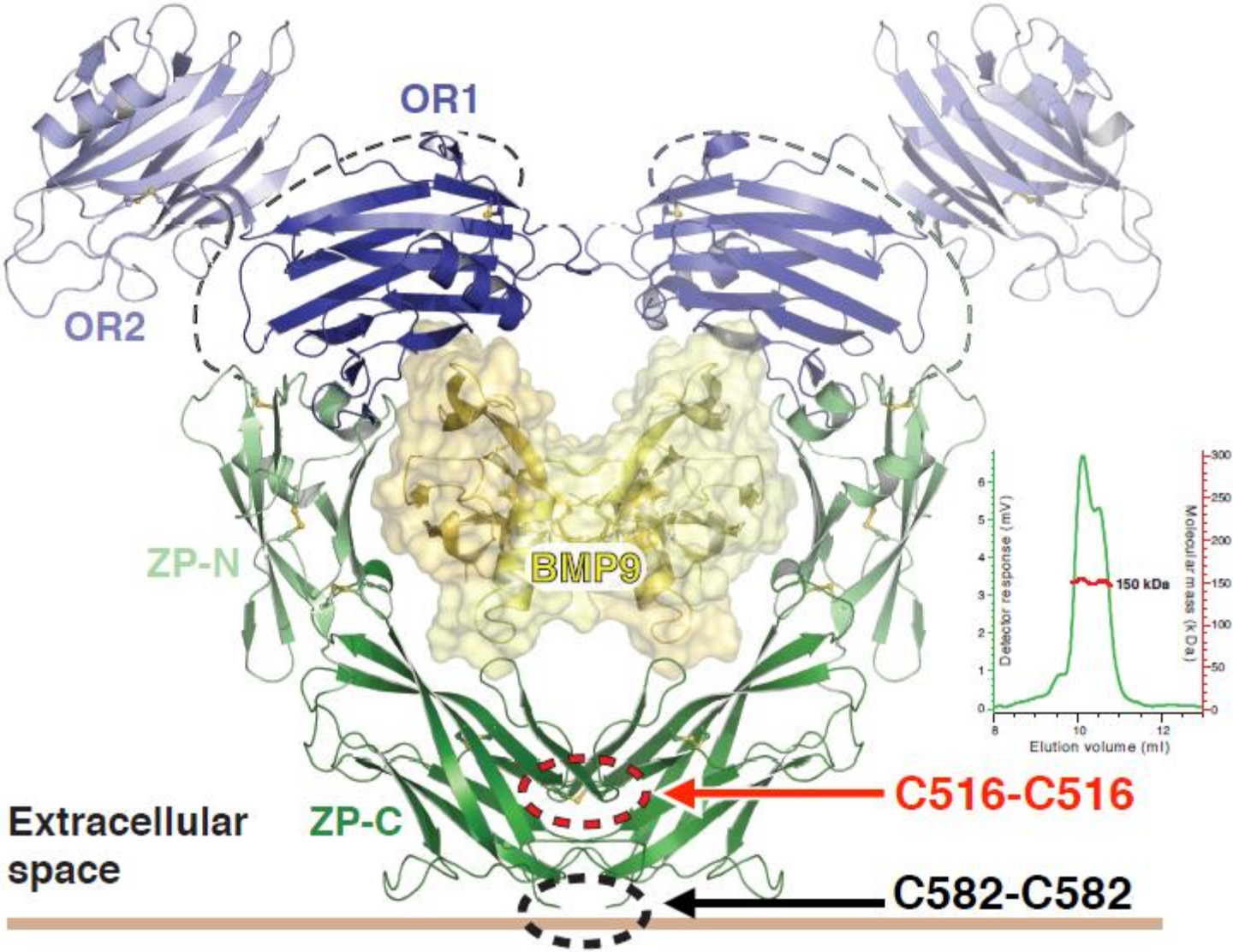
12th HHT INTERNATIONAL SCIENTIFIC CONFERENCE

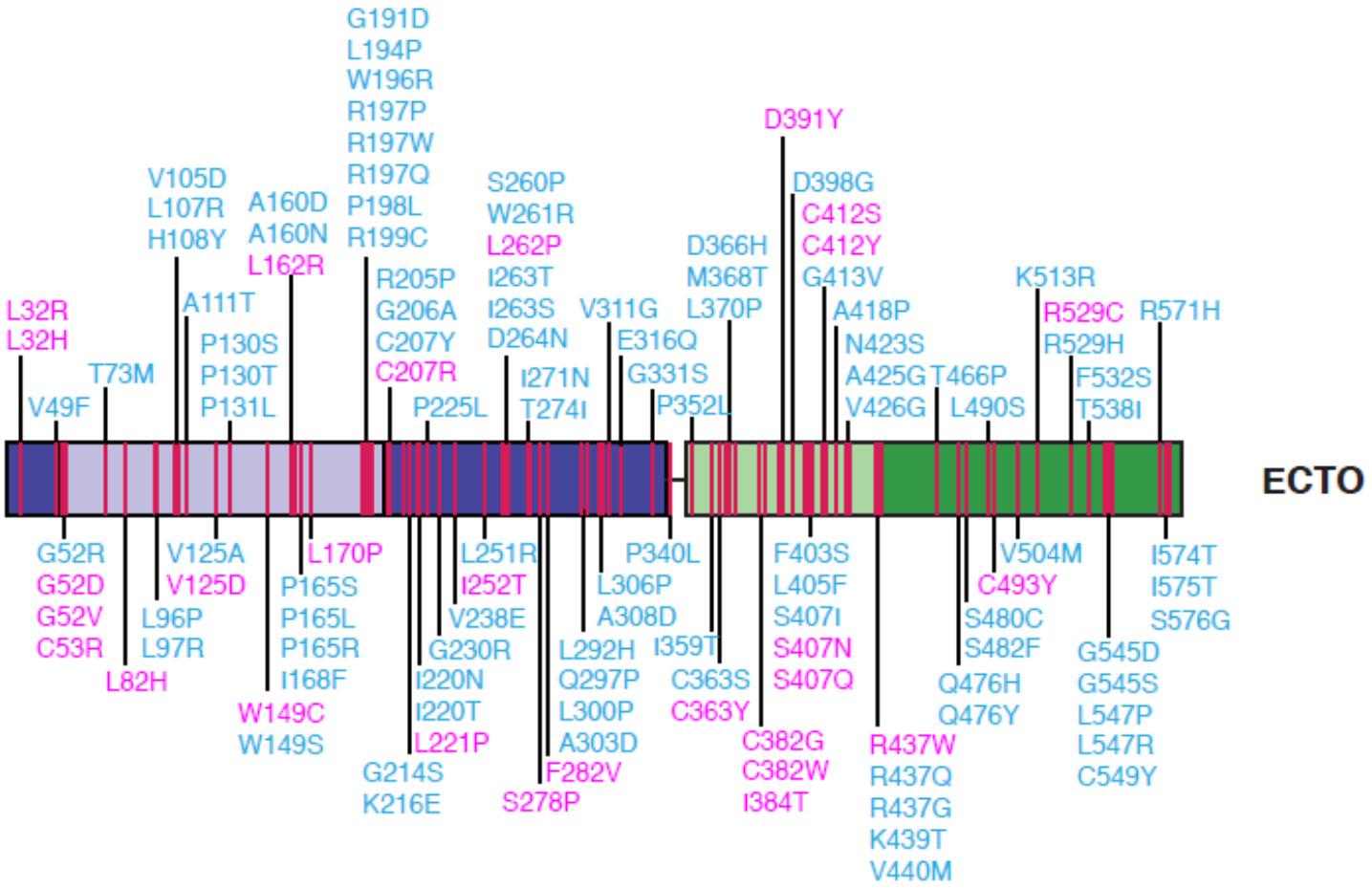
cure hht



The HHT signalling pathway

- 3D structure of endoglin and BMP9


OR1
OR2
ZP-N
BMP9
ZP-C
Extracellular space
C516-C516
C582-C582
Detector response (mV)
Molecular mass (kDa)
150 kDa
Elution volume (ml)


G191D
L194P
W196R
R197P
R197W
R197Q
P198L
R199C
V105D
L107R
H108Y
A160D
A160N
L162R
S260P
W261R
L262P
I263T
I263S
D264N
D391Y
D398G
C412S
C412Y
G413V
D366H
M368T
L370P
K513R
R529C
R529H
R571H
L32R
L32H
A111T
P130S
P130T
P131L
R205P
G206A
C207Y
C207R
V311G
E316Q
G331S
P352L
A418P
N423S
A425G
V426G
T466P
L490S
F532S
T538I
V49F
T73M
P225L
I271N
T274I
ECTO
G52R
G52D
G52V
C53R
L82H
V125A
V125D
L96P
L97R
L170P
P165S
P165L
P165R
I168F
W149C
W149S
L251R
I252T
V238E
G230R
I220N
I220T
L221P
G214S
K216E
P340L
L306P
A308D
L292H
Q297P
L300P
A303D
F282V
S278P
I359T
C363S
C363Y
F403S
L405F
S407I
S407N
S407Q
C382G
C382W
I384T
R437W
R437Q
R437G
K439T
V440M
V504M
C493Y
S480C
S482F
Q476H
Q476Y
I574T
I575T
S576G
G545D
G545S
L547P
L547R
C549Y

- HHT animal models
- Heterozygous
- Inducible KO
- Tissue specific KO – new Smad4
- Combined suppression/overexpression

- Antibodies Anti-BMP9/BMP10 (transmammary delivery)
- Adenoviral vectors (ALK1 mut) brain AVMs
- Zebra fish – endoglin / ALK1

- HHT animal models
- Myeloid lineage specific KO of ENG. Implications in infections
- Endoglin expression in SMCs. CRISPR – siRNA. Implications in mural cell recruitment

- HHT in vitro models
- Vascularized micro-organ (VMO). ECs & vSMCs mimicking arteries-veins network

Role of blood flow in endoglin-dependent signaling:

Flow potentiates BMP9 signaling and promotes endoglin-ALK1 complexes

nature
cell biology

Endoglin prevents vascular malformation by regulating flow-induced cell migration and specification through VEGFR2 signalling

Yi Jin[1], Lars Muhl[1], Mikhail Burmakin[1], Yixin Wang[1], Anne-Claire Duchez[1], Christer Betsholtz[2,3], Helen M. Arthur[4] and Lars Jakobsson[1,5]

Endoglin controls blood vessel diameter through endothelial cell shape changes in response to haemodynamic cues

Wade W. Sugden[1,2], Robert Meissner[2,3], Tinri Aegerter-Wilmsen[4], Roman Tsaryk[1,2], Elvin V. Leonard[1,2], Jeroen Bussmann[1,7], Mailin J. Hamm[1,5,2], Wiebke Herzog[1,5,2], Yi Jin[6], Lars Jakobsson[6], Cornelia Denz[2,3] and Arndt F. Siekmann[1,2,8]

Endoglin moves and shapes endothelial cells

Victoria L. Bautch

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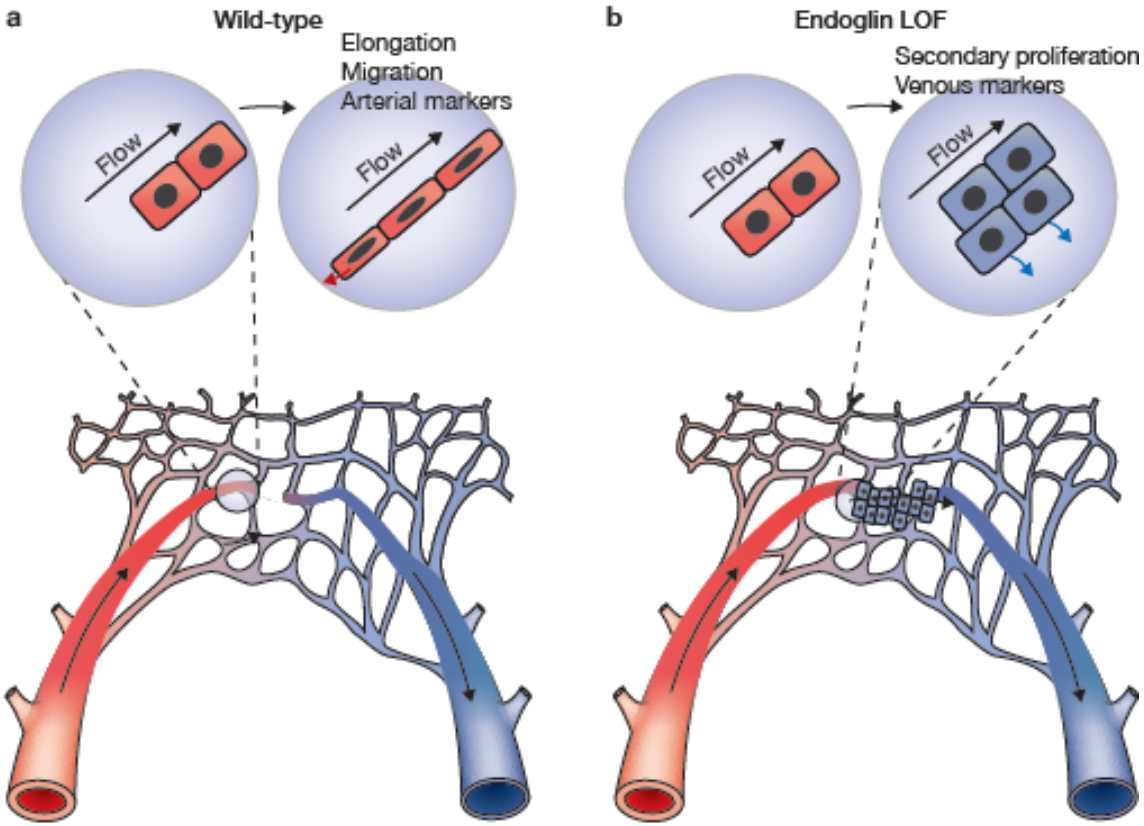


Figure 1 Endothelial cells use endoglin to migrate and remodel vessels. (**a**) Endoglin-mediated processes in wild-type endothelial cells of arterioles lead to migration against the direction of blood flow and cell shape changes that prevent formation of arteriovenous shunts. (**b**) Loss of endoglin (loss-of-function, LOF) prevents migration against the flow direction and cell shape changes, leading to expression of venous markers and secondary proliferation associated with arteriovenous shunts. Black arrows, direction of blood flow; coloured arrows, direction of endothelial cell migration/expansion.

HHT treatments ?

Potential therapeutic targets:

VEGF, PI3K/AKT, TEK/Ang2 (Smad4), CXCR4/CXCL12 (tip Ecs, integrin act.)

Repurposing of drugs:

Tacrolimus (drug screening ID1 reporter-Activator of ALK1). Previous reports on liver transplant & ENG and ALK1 expression. Ongoing Clin. trial

HHT treatments ?

Potential therapeutic strategies:

Gene Therapy (AAV1- blood vessel)

Cell therapy. Induced pluripotent stem cells (iPSCs). Unlimited source combined with rescue of WT

Repurposing of drugs:

Tacrolimus (Activator of ALK1).
Previous reports on liver transplant & ENG and ALK1 expression

Treatment Manifestations of an Endoglin Antibody

TRACON PHARMA

A human HHT model?

Charles Theurer

Currently, **TRC105** is being investigated in multiple clinical trials in combination with other agents, including Avastin® and other VEGF inhibitors. In clinical trials to date, TRC105 has been generally well tolerated as a single agent and in combination with VEGF inhibitors. In addition, Santen Pharmaceutical Co., Ltd., is developing TRC105 as a treatment for wet age-related macular degeneration ("AMD"), the leading cause of blindness in the western world.

Treatments of antigiogenic drugs related to the HHT signaling pathway reproduce HHT symptoms (epistaxis, telangiectasias, gingival bleeding):

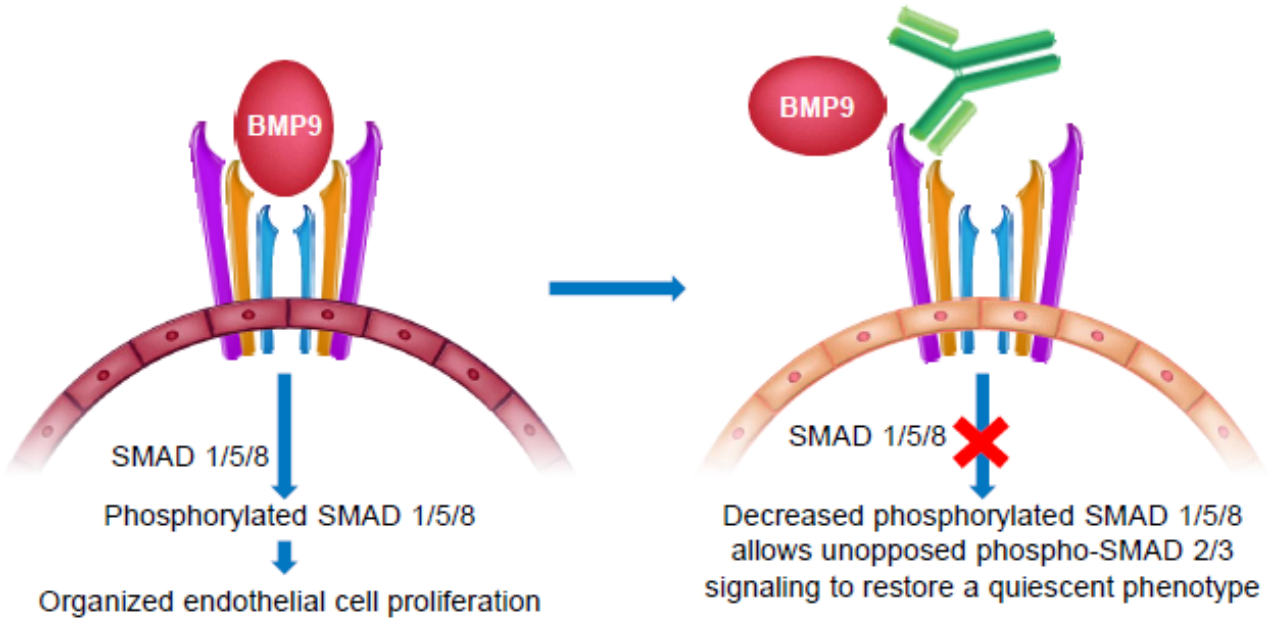
TRC105 (ENG)

Dalantercept (BMP9/10)

PF03446962 (ALK1)

TRC105: Endoglin Antibody

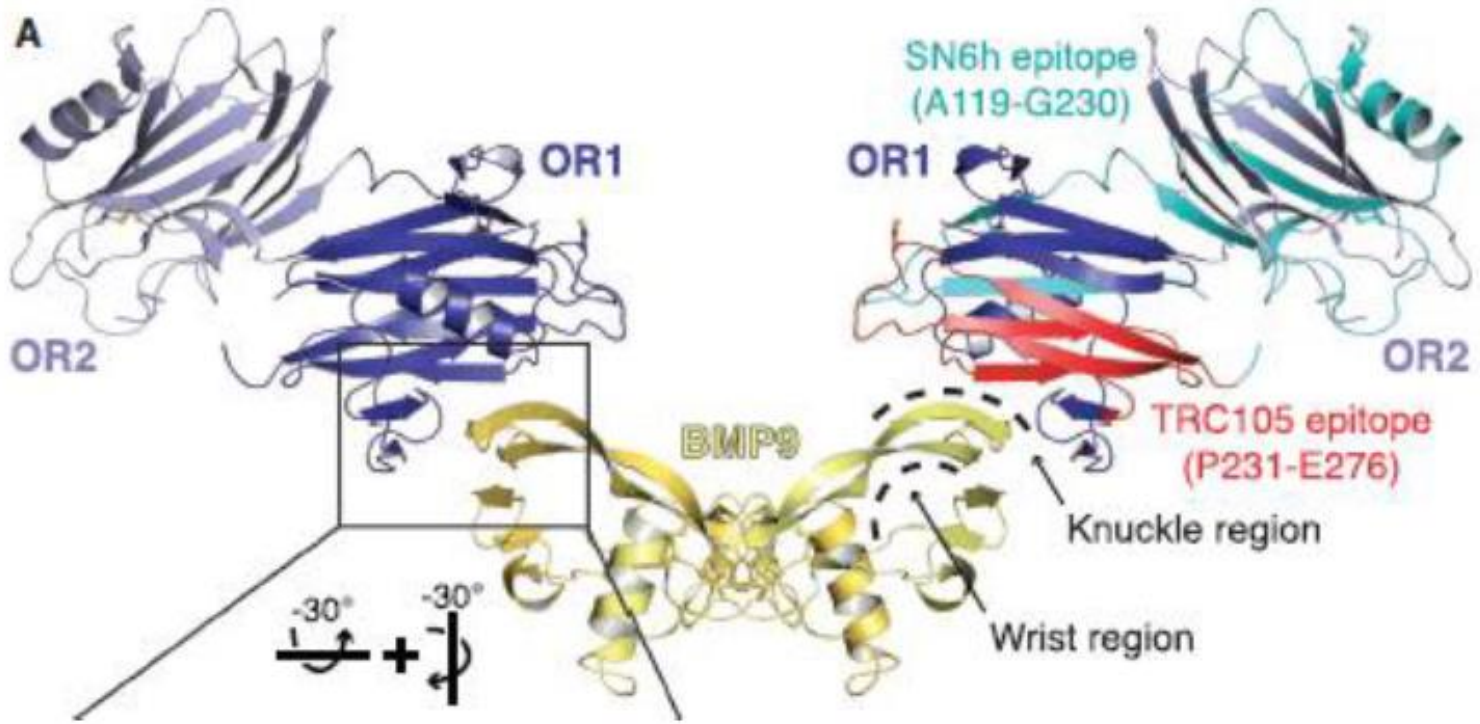
- TRC105 binds a precise endoglin epitope to inhibit BMP binding
- TRC105 also potently mediates ADCC



TRACON PHARMA

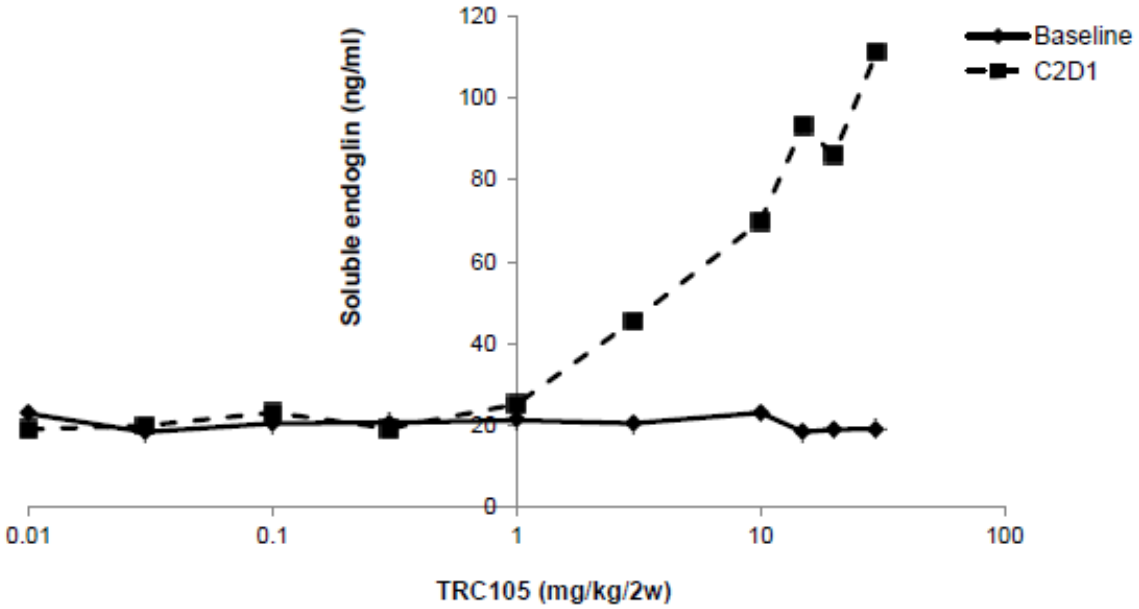
Nolan-Stevaux 2012; Saito 2017

The Precise Endolgin-BMP9 Interaction has been Elucidated by Crystallography and Overlaps with the TRC105 Binding Site



TRACON Saito et al 2017

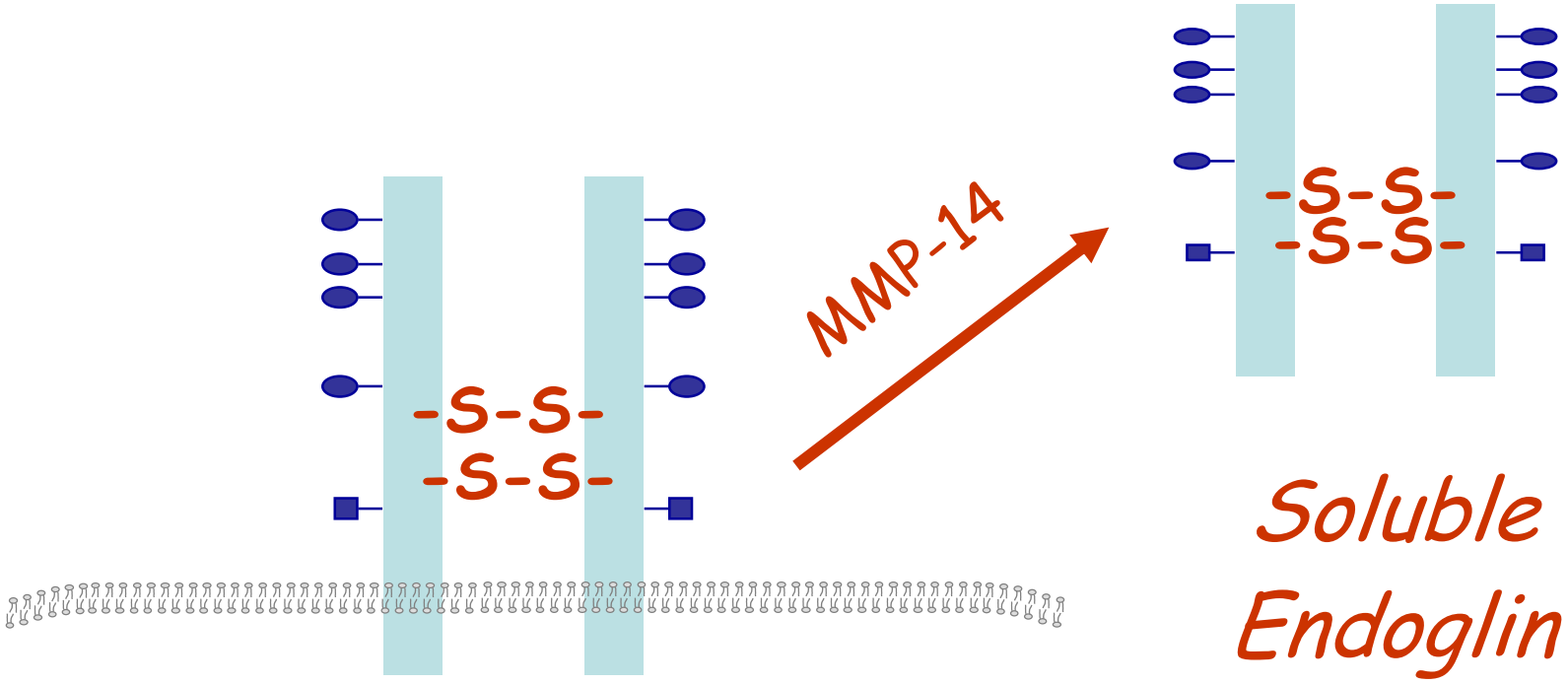
Dose Dependent Soluble Endoglin in Phase 1 Oncology Patients



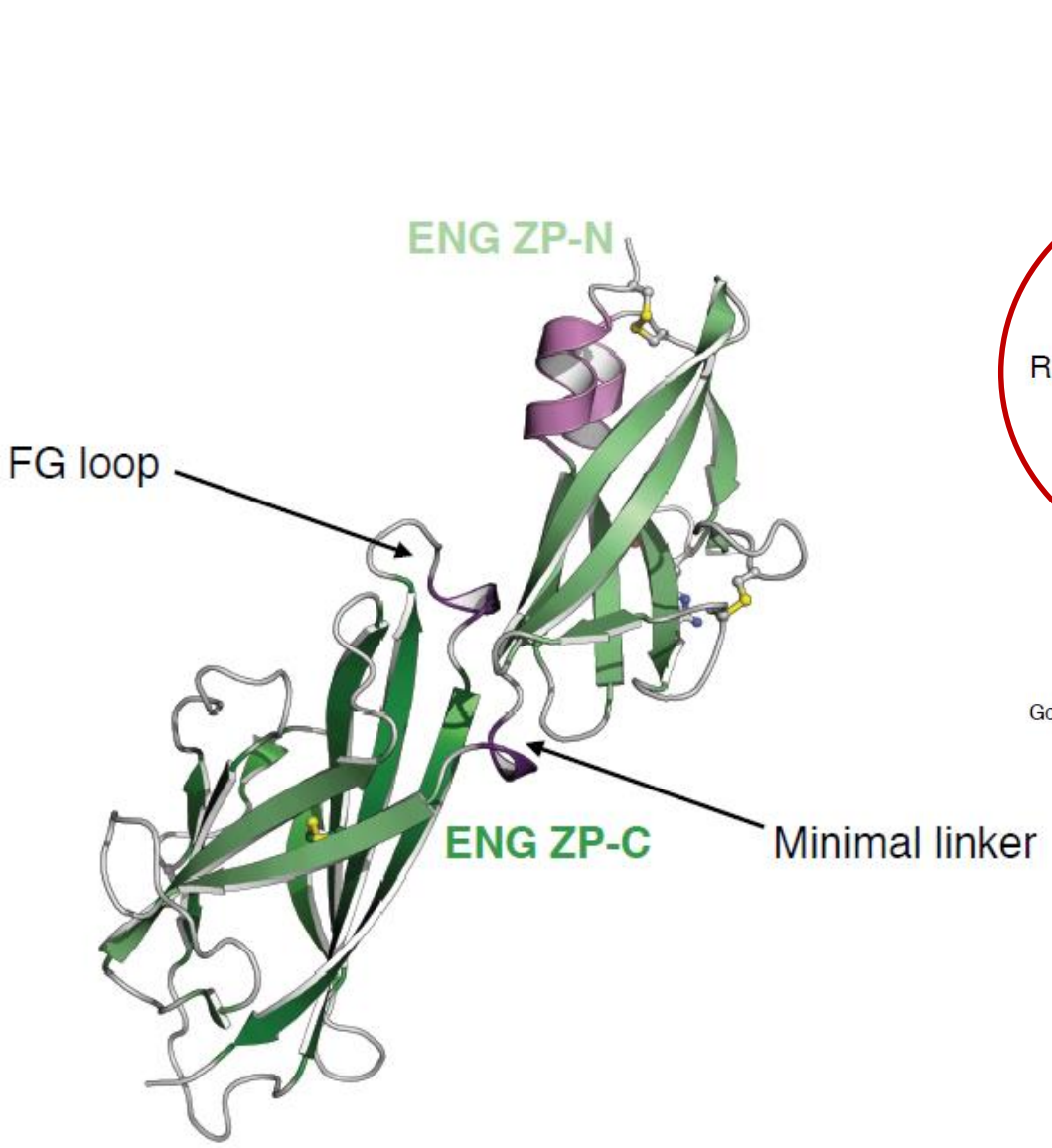
Shedding of endoglin through is an additional mechanism of action of TRC105, as shed endoglin is antiangiogenic in preclinical models by sequestering the endoglin ligand BMP9

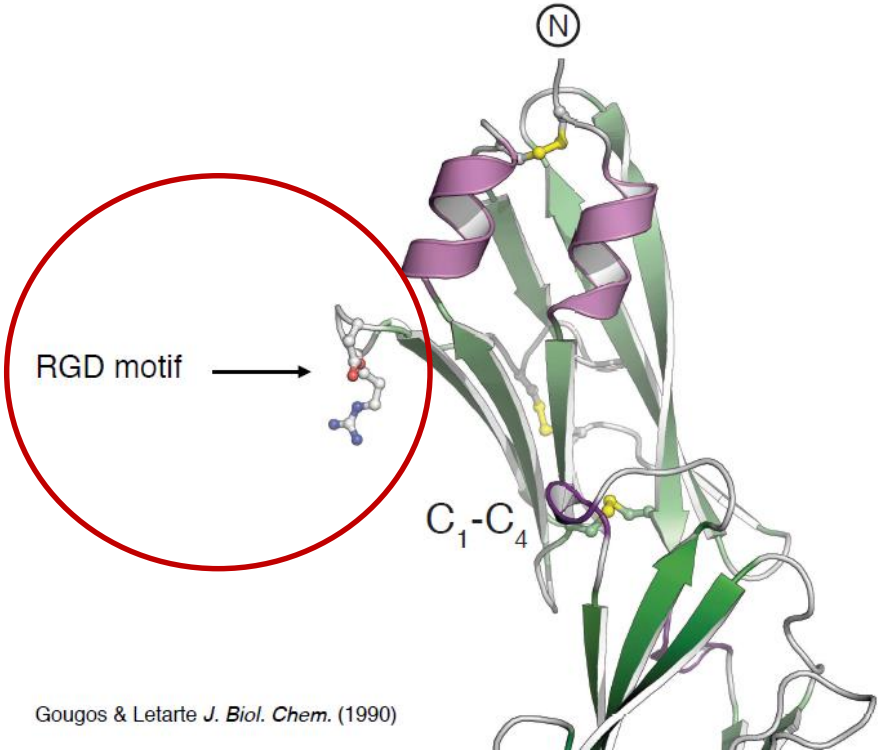
TRACON PHARMA

Release of soluble Endoglin



Role of endoglin in integrin-mediated cell adhesion





Gougos & Letarte *J. Biol. Chem.* (1990)

HHT Genetics:

-Exon and intron flanking regions of known HHT genes

-Identify new mutations in non-coding regions –intronic regions, promoters, regulatory regions

-NGS and RNAseq to identify new HHT genes and new gene modifiers

Session 7A Workshop:
ALK1 & Endoglin signalling in HHT

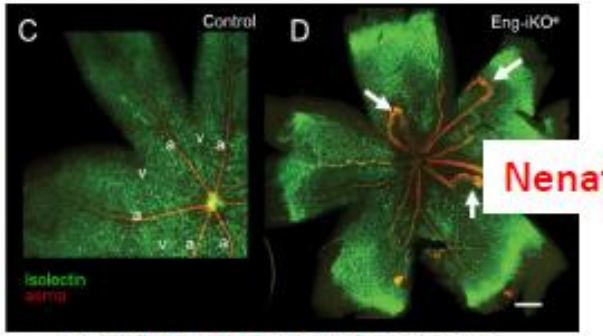
Friday, June 9

1:15pm – 2:45pm

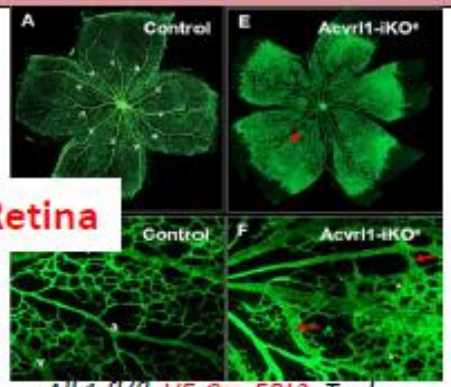
Co-Chairs: Helen Arthur and Franck Lebrin

cure hht

Q8 Are the pre-clinical models of HHT relevant?

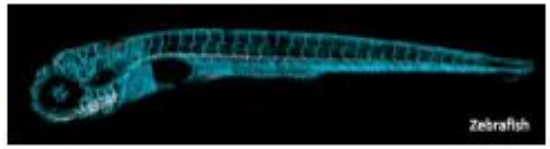


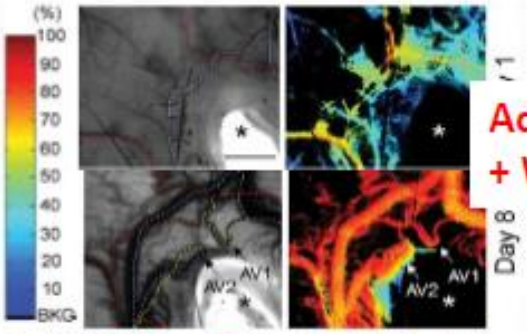
Nenatal Retina



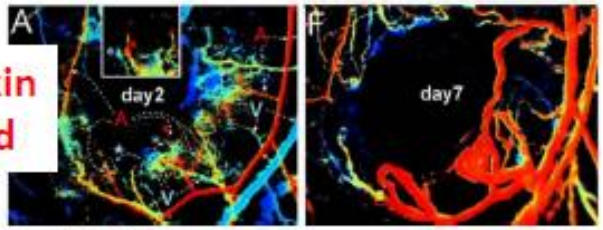
Engfl/fl; VE-Cre-ERt2 Mahmoud et al Circ Res 2010

Alk1 fl/fl; VE-Cre-ERt2; Tual-Chalot et al PlosOne 2014



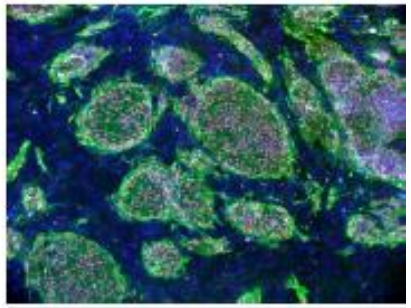


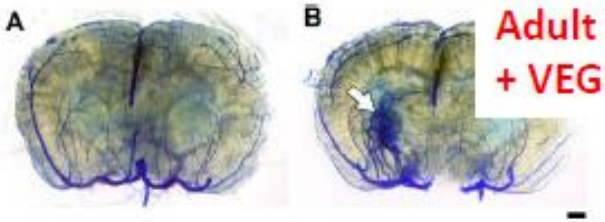
Adult Skin + Wound



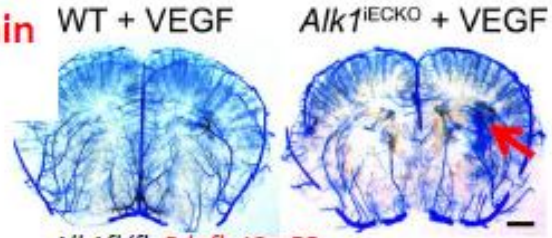
Engfl/fl; Scl-Cre-ER Garrido-Martin et al, ATVB, 2014

Alk1fl/fl; R26Cre-ERt2; Han et al Angiogenesis , 2014





Adult Brain + VEGF



Eng	zfl/zfl	zfl/zfl
Cre	+	+
TM	+	+
VEGF	+	+

Engfl/fl; R26Cre-ERt2+ AAV-VEGF Choi et al, PlosOne, 2014

Alk1fl/fl; Pdgfb-iCreER + AAV-VEGF

Chen et al Stroke ,2014

Conclusions-take home messages from workshop

Signalling in endothelial & perivascular cells
Q1 Does TGFbeta signalling have a role in HHT?
Q2 Is BMP9 anti-angiogenic or pro-angiogenic?
Q3 Is HHT an abnormal response to angiogenic signals?
Q4 Do ENG and ALK1 have same or different roles?
Q5 VEGF signalling and HHT – is increased VEGF signalling a primary or secondary response?
Q6 Is there a role of the Notch Pathway in HHT?
Q7 What is the impact of HHT modifiers?
Q8 Are the HHT pre-clinical models relevant?