CENTRE DE RECHERCHE EN AUTOMATIQUE DE NANCY

Intravital microscopy for imaging brain tumors xenografts

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AM2I

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Workshop « Systèmes modèles précliniques en cancérologie »

Cancéropôle Grand Est, Strasbourg, 15 novembre 2019

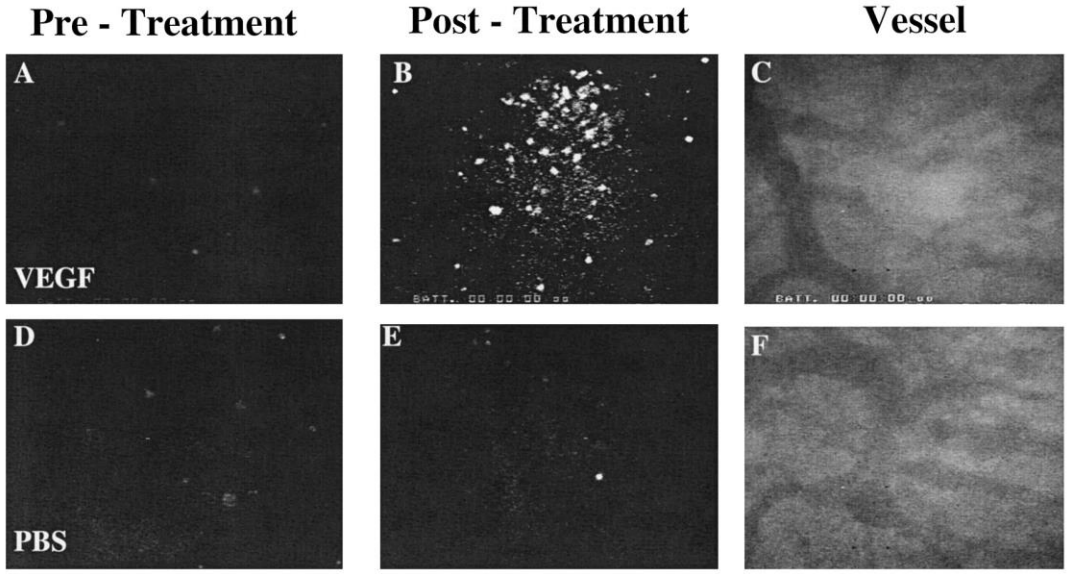
cnrs UMR 7039 UNIVERSITÉ DE LORRAINE

Institut de Cancérologie de Lorraine Alexis Vautrin
Ensemble, construisons l'avenir

THE BEGINNING OF INTRAVITAL MICROSCOPY IN 90'S

- IVM allows the achievement of two key tasks in tumor biology: the behavioral observation of cells or tissues within the real environment and the quantification of biological relevant phenomena.
- The principe is relatively easy = open a window through the tissues to visualize the cancer cells or tissue environment with a follow-up in time by a microscopic approach

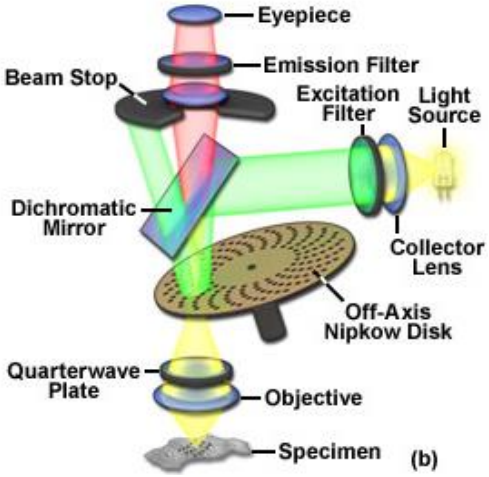
  ➔ initially limited by the resolution of microscopes

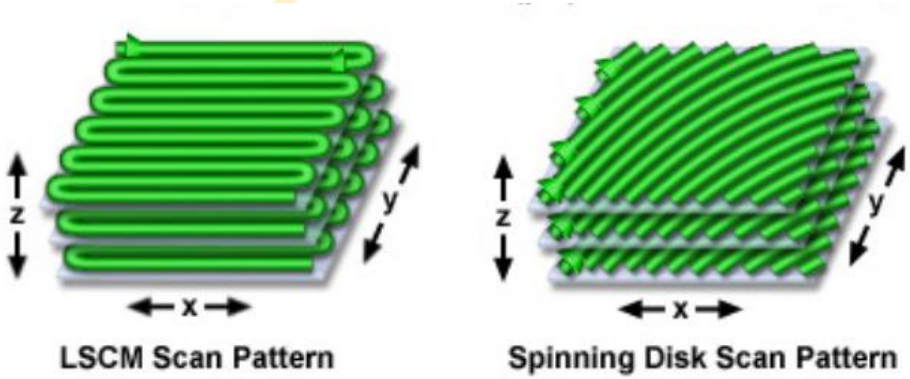


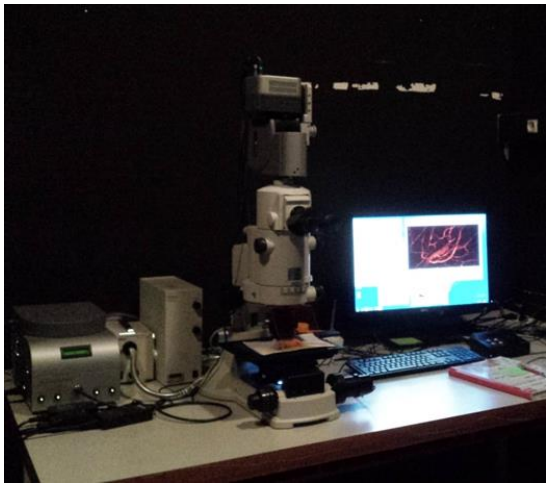
WAYNE ET AL, CANCER RES 1992

THE PROGRESS OF OPTICAL MICROSCOPY

Imaging techniques	Explanations
Spinning disk confocal microscopy	acquire high-resolution images rapidly, imaging up to a depth of <100 µm, with low photobleaching and toxicity.
Single-photon confocal microscopy	high temporal and spatial resolution, but with a limited tissue penetration (50–60 µm) and photobleaching and toxicity.
multi-photon confocal microscopy	offer a good penetration depth, although this is restricted to 800–1000 µm in soft tissues (e.g. brain) and up to 200 µm in hard tissues (e.g. bone), with low photobleaching and toxicity
Optical coherence tomography (OCT)	A noninvasive optical signal acquisition and processing method that can be used to generate micrometer-resolution, three-dimensional images at depth.

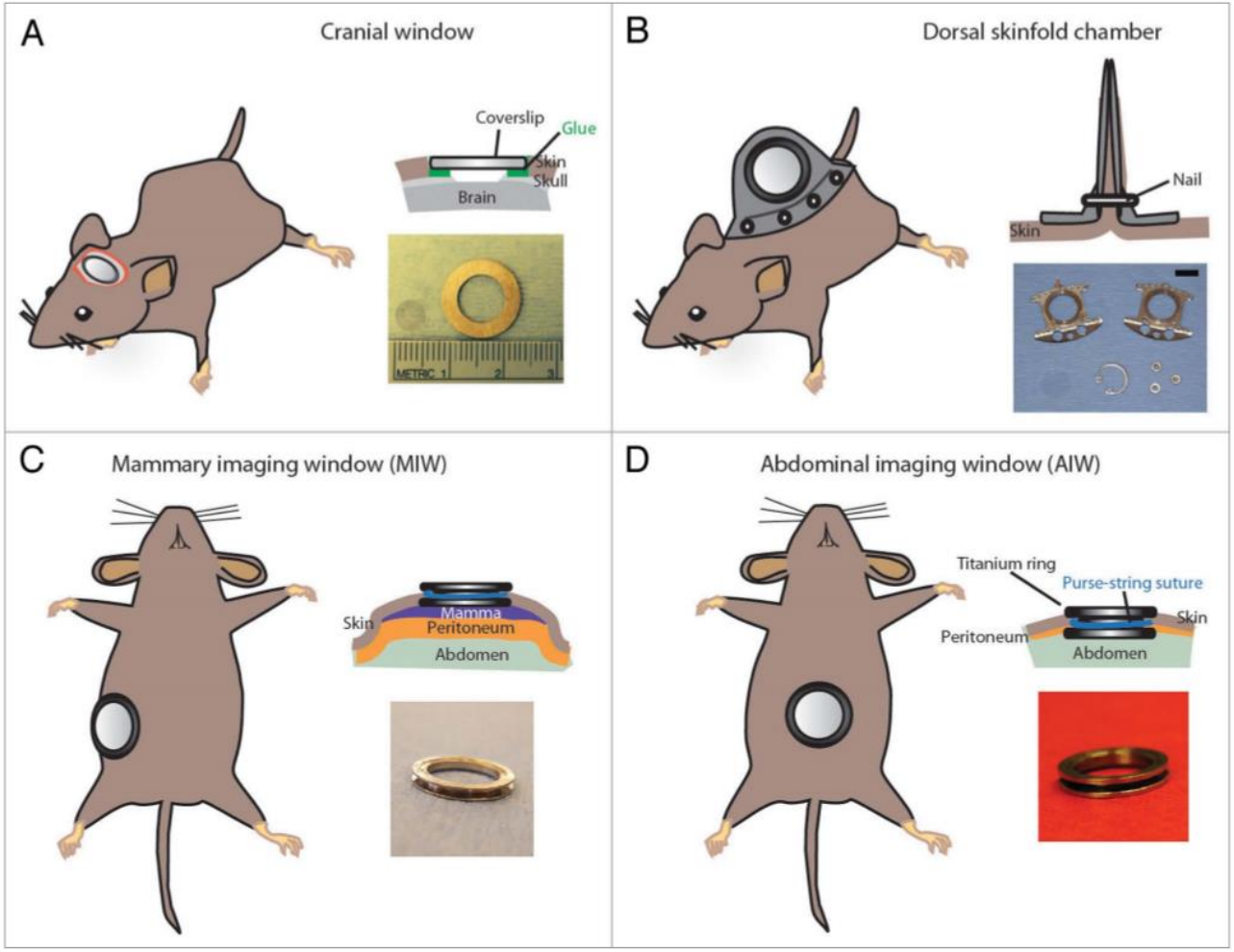






MACROSCOPE NIKON AZ100 WITH SPINNING DISK (ANDOR TECHNOLOGY, CRAN)

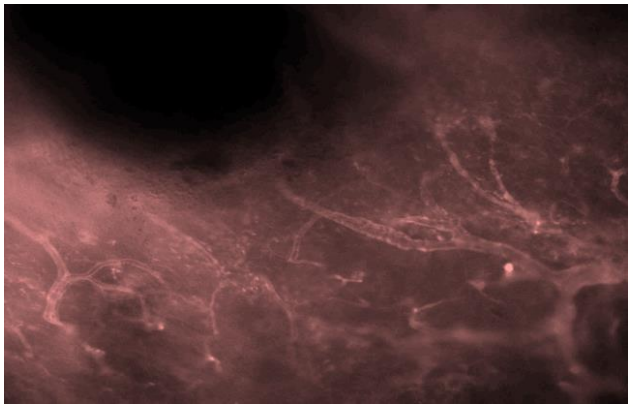
THE DIFFERENT WAYS OF VISUALIZATION OF TUMORS



ALIEVA ET AL, INTRAVITAL 2014

WHAT CAN BE VISUALIZED WITH INTRAVITAL MICROSCOPY ?

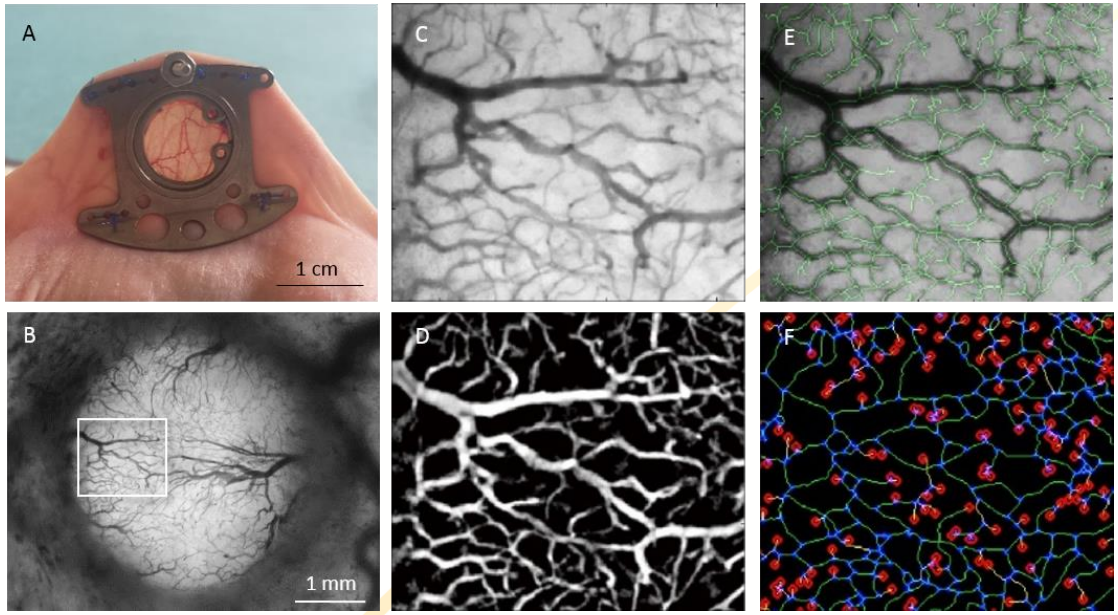
parameters	Molecular probes
Tracking cancer cells or other cells (i.e. leukocytes)	GFP, RFP, calcein, DiI, DiO, nano-crystals (Qdots)
Tumor size	Endogenous contrast, GFP, OCT
Vascular architecture (diameter, length, surface area, volume, branching patterns)	Endogenous contrast, dextran, nano-objects
Extracellular matrix	Second harmonic generation (type I collagen)
Blood flow rate	dextran, RBC (fluorescent, endogenous contrast), OCT
Vascular permeability	BSA, low PM dextran, nano-objects,



PERITUMORAL VASCULATURE (DEXTRAN) NIKON AZ100

FOLLOW-UP OF TUMOR SIZE AND ANGIOGENESIS BY TRANS-ILLUMINATION WITH DORSAL SKINFOLD CHAMBER

DSC | Intravital microscopy | skeleton



Tumor fragment of U87-MG

Segmentation

Quantification

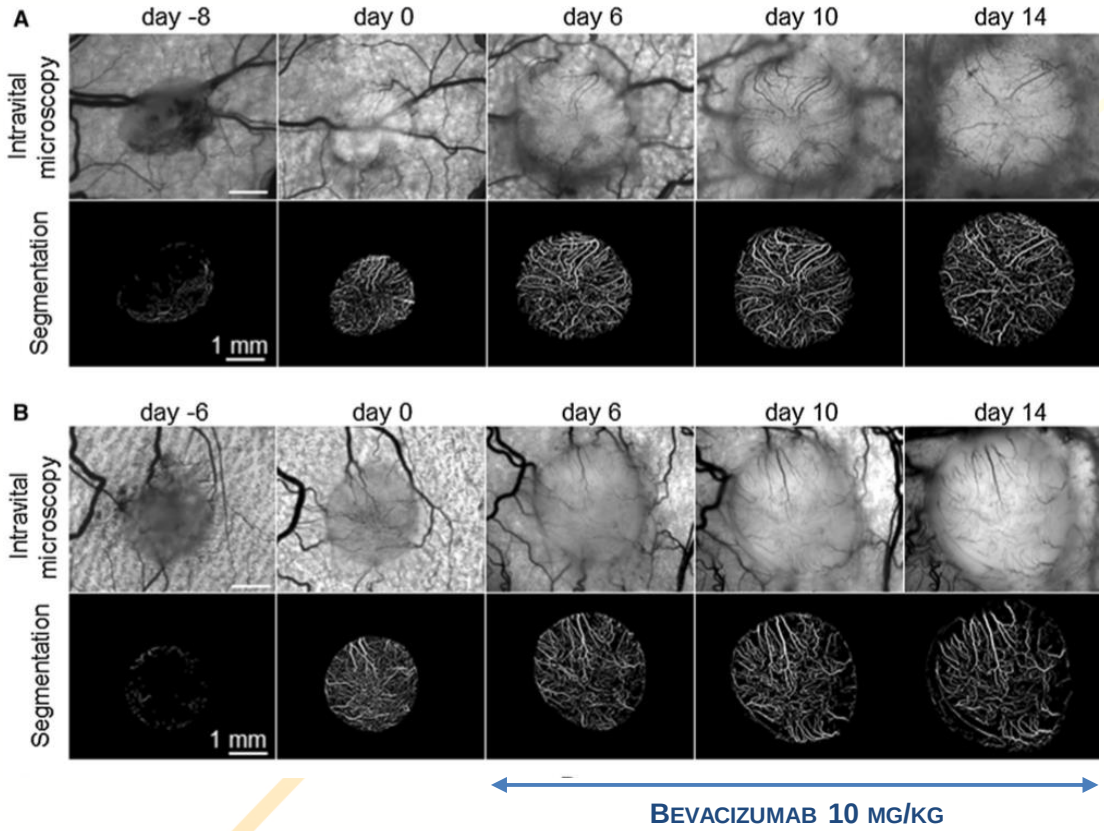
Length of vessel
number of junctions

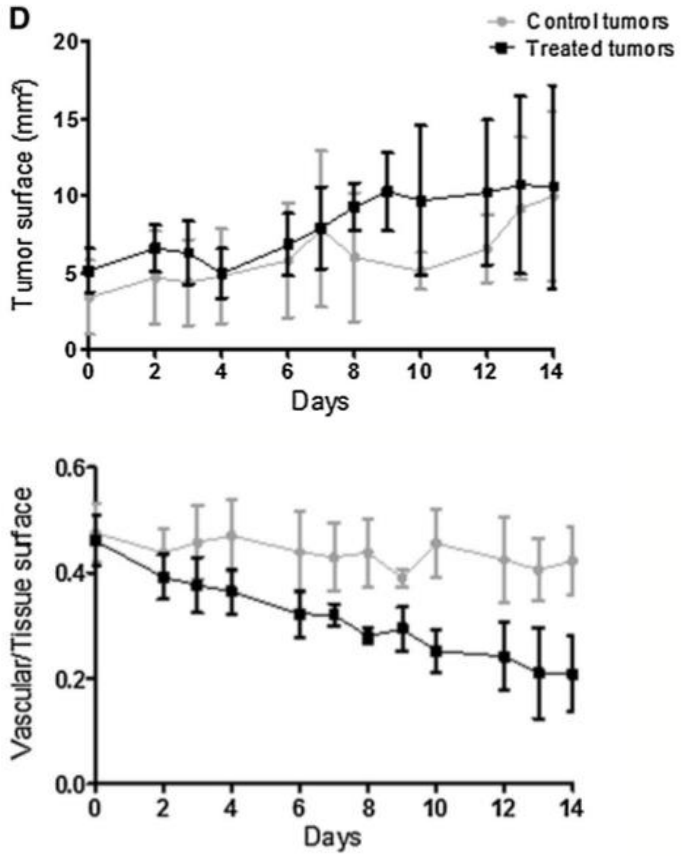
OBJECTIVES OF THE STUDY :

1) DEFINE AN ALGORITHM TO QUANTIFY ANGIOGENESIS WITH TRANS-ILLUMINATION OF THE TUMOR
2) DEFINE THE BEST PERIOD TO ASSOCIATE A THERAPY (CHIMIOTHERAPY, RADIOTHERAPY OR PHOTODYNAMIC THERAPY) WITH BEVACIZUMAB TREATMENT

EL ALAOUI ET AL, ANGIOGENESIS 2017

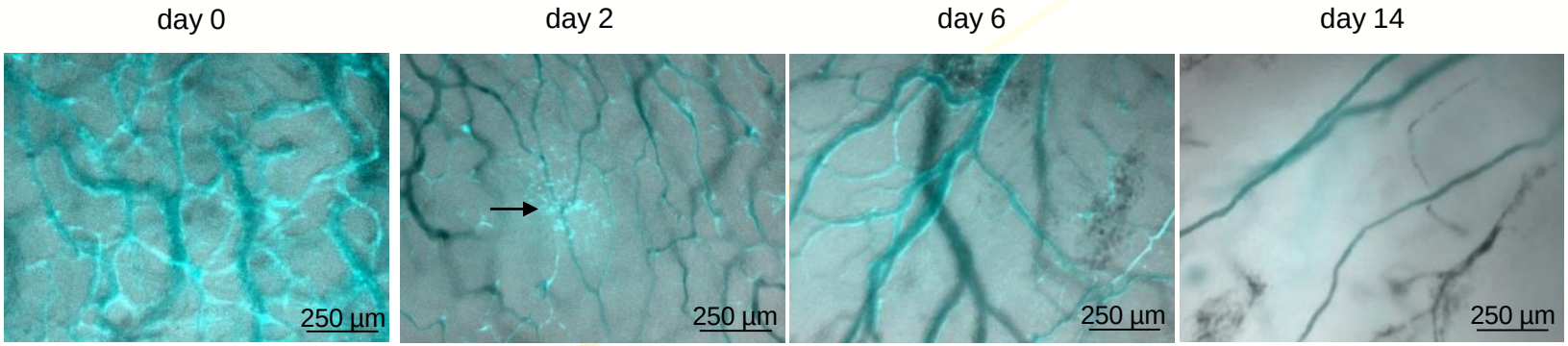
FOLLOW-UP OF TUMOR SIZE AND ANGIOGENESIS BY TRANS-ILLUMINATION WITH DORSAL SKINFOLD CHAMBER





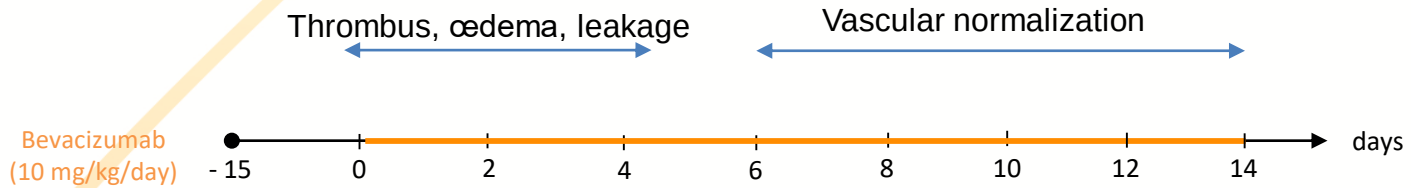
EL ALAOUI ET AL, ANGIOGENESIS 2017

FOLLOW-UP OF VASCULAR PERMEABILITY WITH DORSAL SKINFOLD CHAMBER



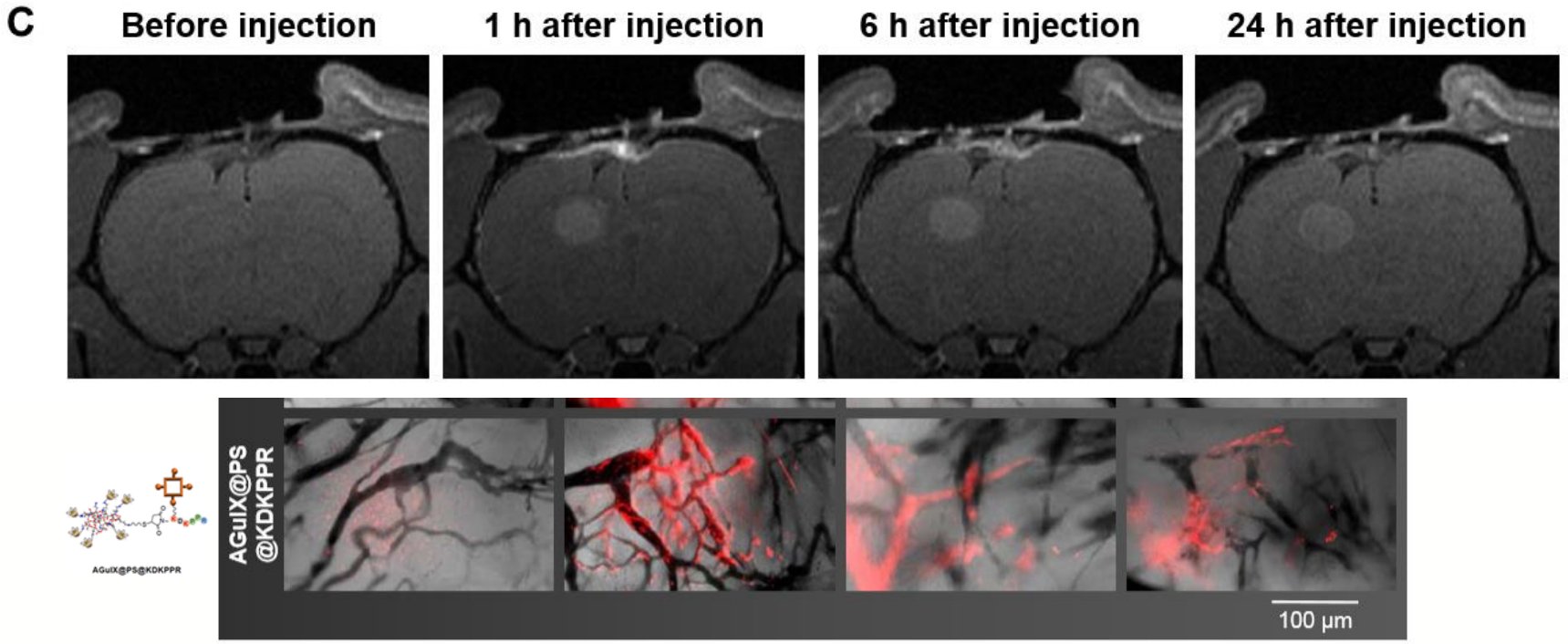
vascular permeability (assessed with fluorescent dextran of 70 kDa) increased between day 2 and 5 to disappear after 6 days of treatment with bevacizumab

➔ define the best period to evaluate efficacy of bevacizumab association



EL ALAOUI ET AL, ANGIOGENESIS 2017

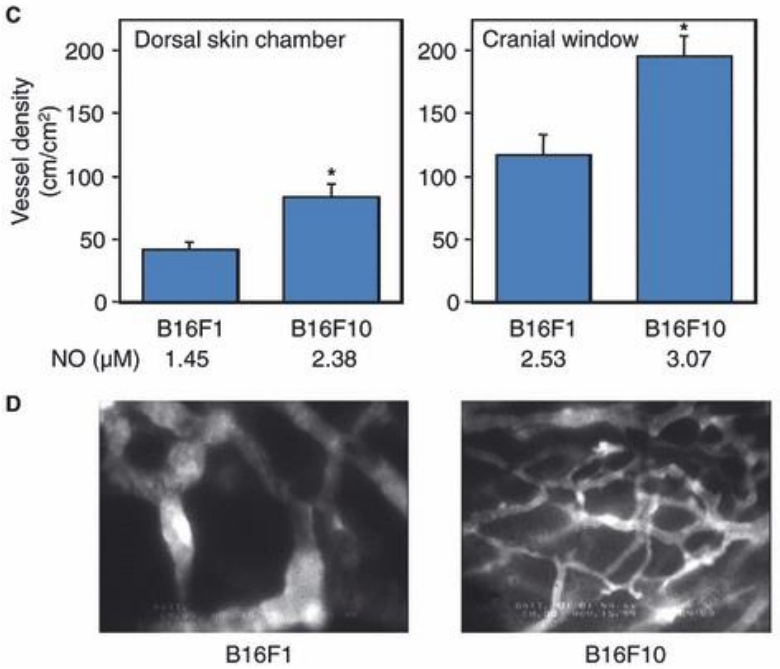
FOLLOW-UP OF NANOPARTICLES TUMOR DISTRIBUTION WITH DORSAL SKINFOLD CHAMBER



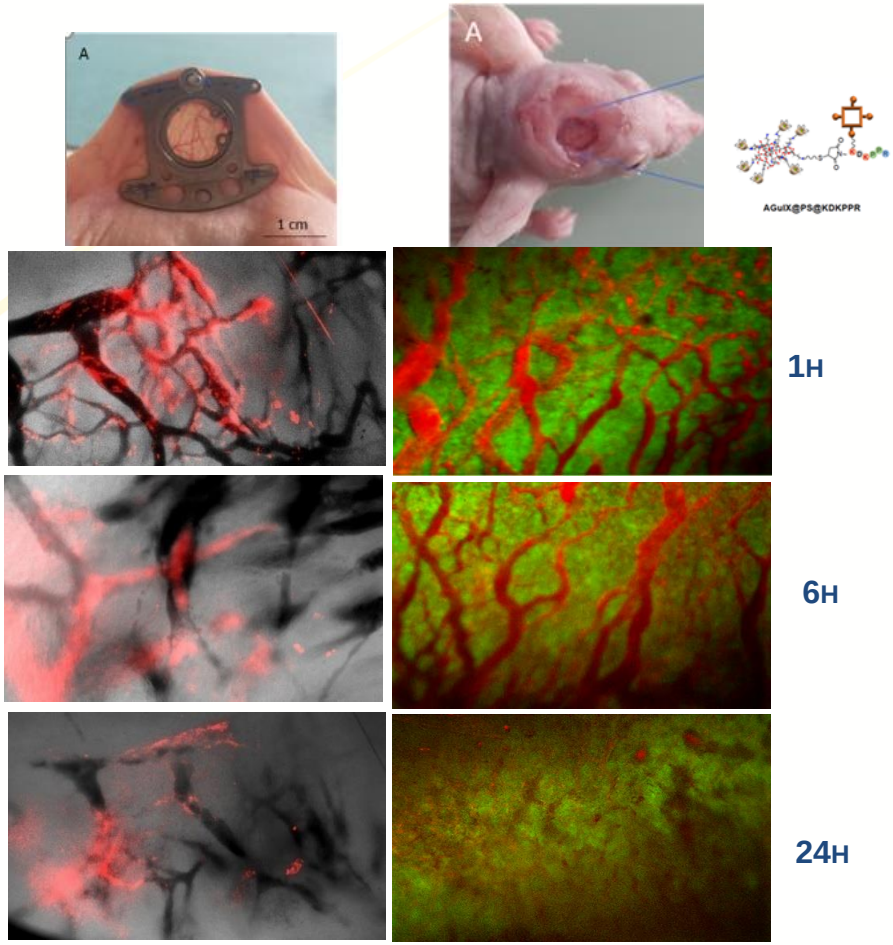
THOMAS ET AL, INT J NANOMEDICINE 2017

- **Vascular persistence of NPs targeting NRP-1 during 24h**

DORSAL SKINFOLD CHAMBER VS CRANIAL WINDOW



FUKURAMA ET AL, MICROCIRCULATION 2010



THESIS WORK OF M. GRIES, CRAN

Cranial window model give a better environment for brain tumors

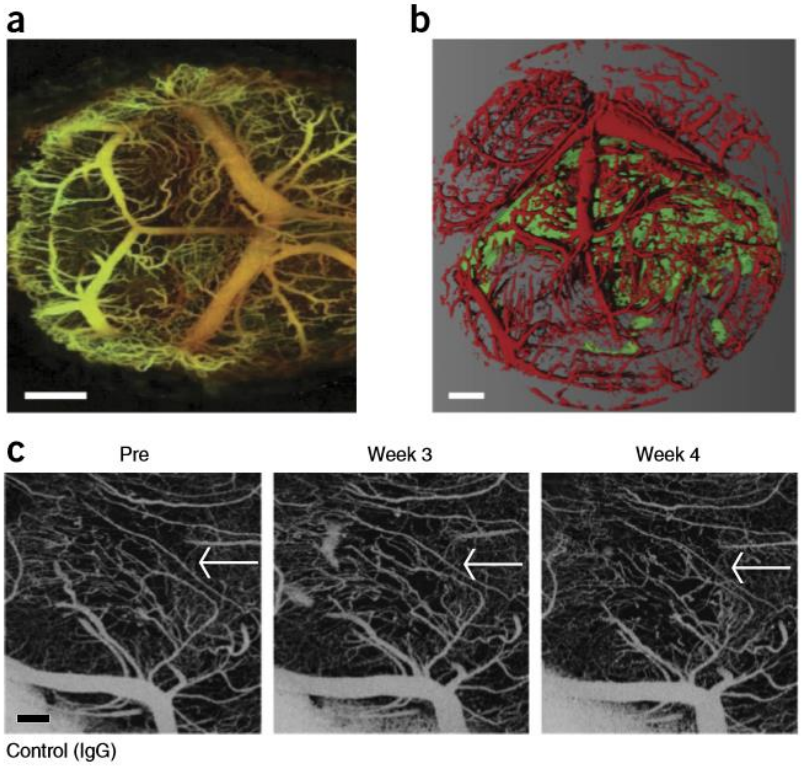
OCT COMBINED WITH CONFOCAL MICROSCOPY FOR IVM

NATURE PROTOCOLS | VOL.12 NO.11 | 2017 | 2251

A cerebellar window for intravital imaging of normal and disease states in mice

Vasileios Askoxylakis[1,6,7], Mark Badeaux[1,2,7], Sylvie Roberge[1], Ana Batista[1,3], Ned Kirkpatrick[1,4], Matija Snuderl[1,5], Zohreh Amoozgar[1], Giorgio Seano[1], Gino B Ferraro[1], Sampurna Chatterjee[1], Lei Xu[1], Dai Fukumura[1], Dan G Duda[1] & Rakesh K Jain[1]

[1]Edwin L. Steele Laboratories, Department of Radiation Oncology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA. [2]Aeglea Biotherapeutics, Austin, Texas, USA. [3]Cell Press, Cambridge, Massachusetts, USA. [4]Novartis Cambridge, Massachusetts, USA. [5]Department of Pathology, New York University Langone Medical Center and Medical School, New York, New York, USA. [6]Present address: Merrimack Pharmaceuticals, Cambridge, Massachusetts, USA. [7]These authors contributed equally to this work. Correspondence should be addressed to R.K.J. (jain@steele.mgh.harvard.edu).



ZEBRAFISH: A MODEL TO CONSIDER FOR IVM OF BRAIN TUMORS

Oncogene (2015) **34,** 1736–1742
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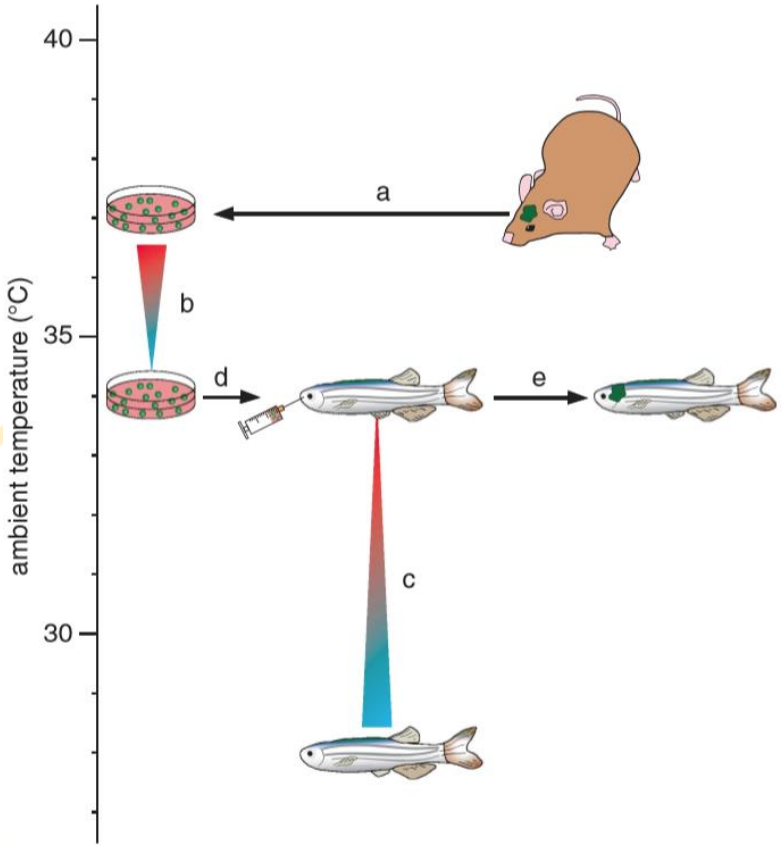
www.nature.com/onc

SHORT COMMUNICATION

Orthotopic models of pediatric brain tumors in zebrafish

CJ Eden[1], B Ju[2], M Murugesan[1], TN Phoenix[1], B Nimmervoll[1], Y Tong[1], DW Ellison[3], D Finkelstein[4], K Wright[5], N Boulos[1], J Dapper[1], R Thiruvenkatam[1], CA Lessman[6], MR Taylor[2] and RJ Gilbertson[1]



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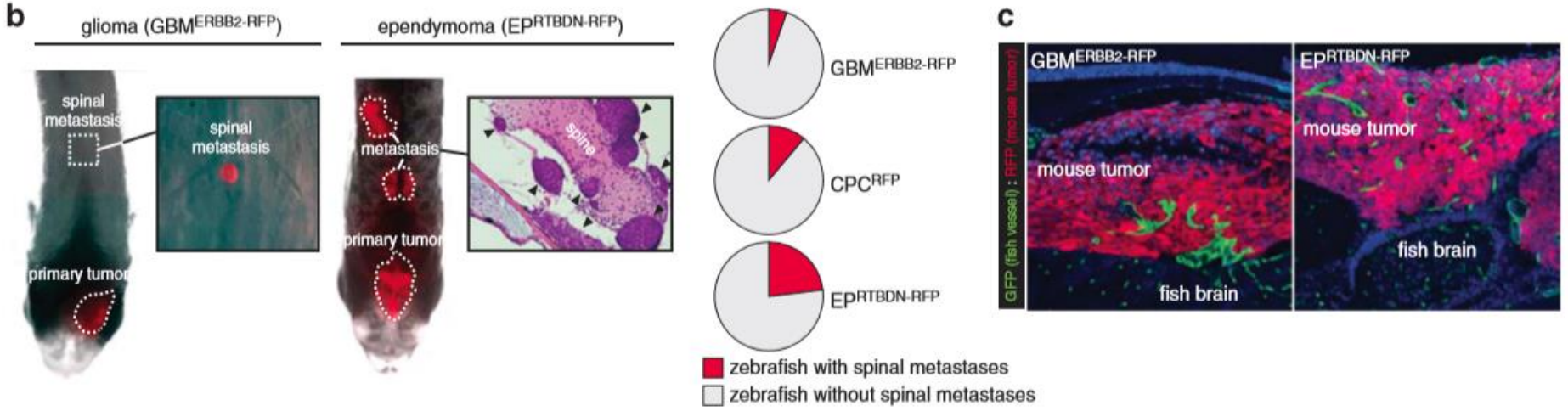

THANK YOU FOR YOUR ATTENTION

QUESTIONS ?