Imaging and interpretation of radiobiological processes

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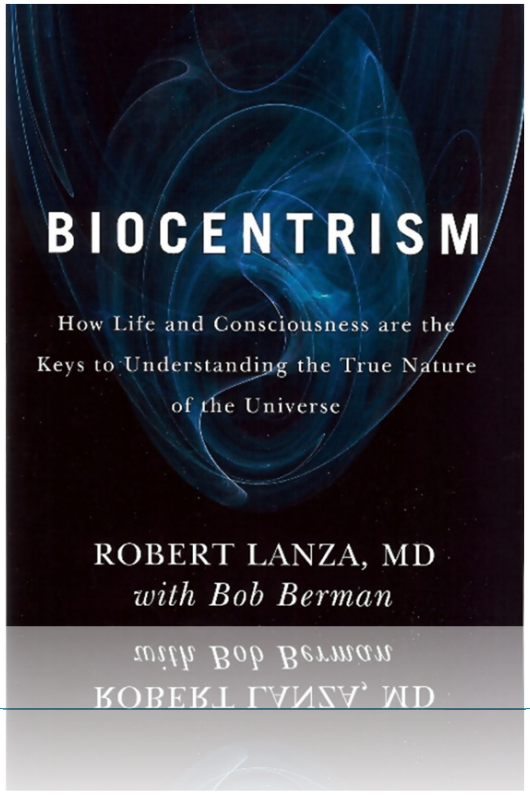
Imaging and biology



Imaging*target matrix

Direct | Surrogate

Targets

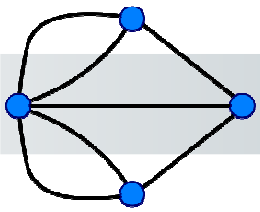
*

Direct | Surrogate

Biomarkers

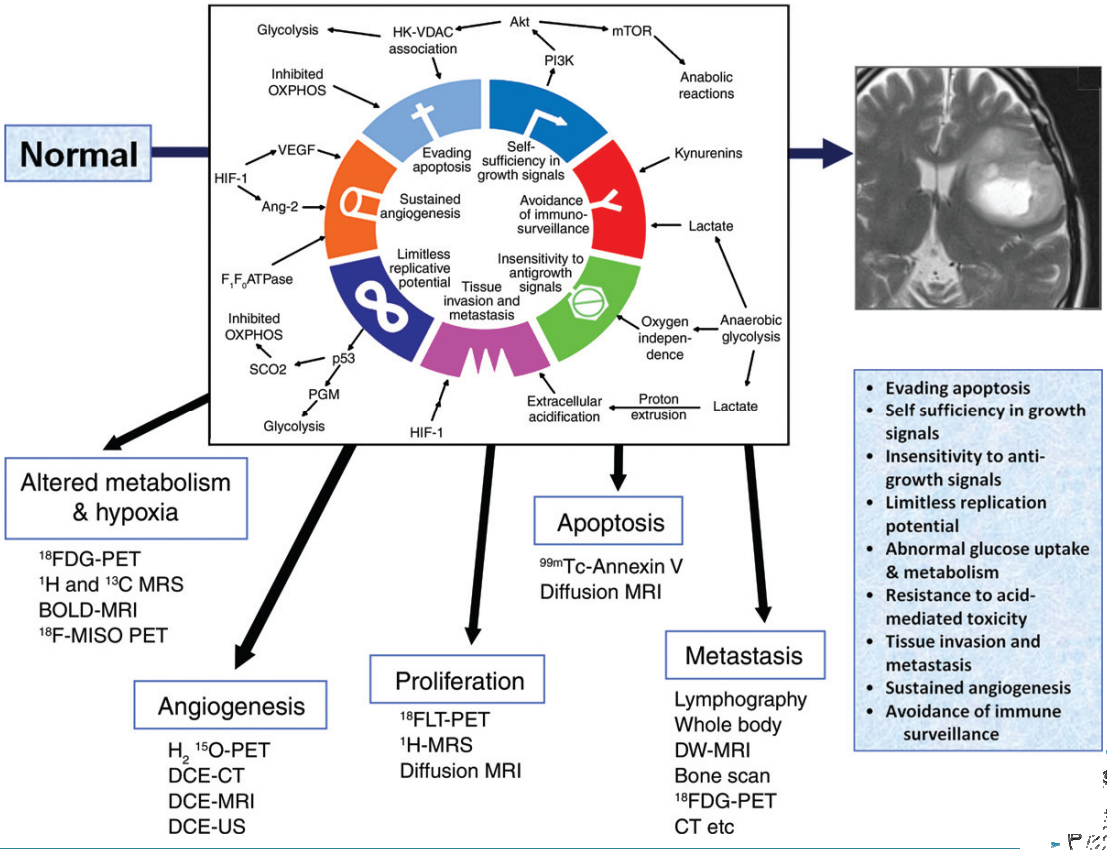
Biological targets

- Hypoxia Direct
- Angiogenesis Direct
- Interstitial fluid pressure (IFP)
- Cell proliferation Direct
- Apoptosis/Necrosis Direct
- Tumor stroma



Imaging “weapons”

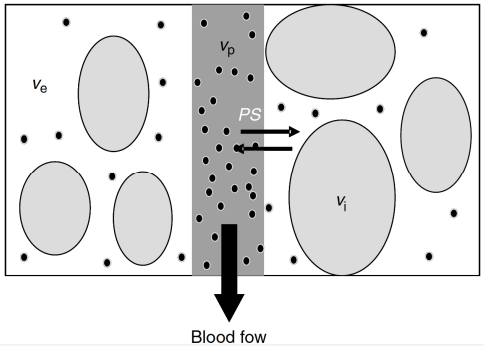
- PET:
  - FDG — Direct
  - MISO — Direct
  - FLT — Direct
- MRI:
  - DCE/perfusion MRI
  - DWI
  - IVIM DWI



Padhani 2010

dynamic relaxivity contrast enhanced MRI (DCE-MRI)

- noninvasive quantitative method
- investigates microvascular structure and function by tracking the pharmacokinetics of injected Gd contrast agents as they pass through the tumour vasculature
- the technique is sensitive to alterations in
  - vascular permeability (Ktrans)
  - extracellular extravascular volume (ve, Fis)
  - vascular volume (BV)
  - blood flow (BF)



DWI-MRI → tissue cellularity, extracellular space tortuosity, and integrity of cellular membranes

- water motion in tissues → modified by
  - flows within conduits (for example, blood vessels, glandular ducts, etc.);
  - interactions with cellular components (hydrophobic phospholipid-containing cellular membranes, intracellular organelles, and macromolecules)
- DW "made" sensitive to large/small displacements of water:
  - Large → macroscopic flows (**low b-values <50–100 s/mm^2**),
  - Small → microscopic extracellular space/intracellular water displacements (**high b-values**)
- DW gradients to standard T2-w sequences (b-values)
- ADC measures water motion restriction (high ADC → low restriction; low ADC → high restriction)

DWI IVIM

- DWI signal is influenced by
  - a fast component due to arteriolar blood flow [Lemke et al 2009]
  - A slow component due to interstitial water diffusion
- Biexponential analysis of DWI signals allows perfusion- from diffusion-effect to be separated
- Perfusion is described by ***f*** *(perfusion fraction)* and ***D**** *(pseudodiffusion coefficient)*

IFP

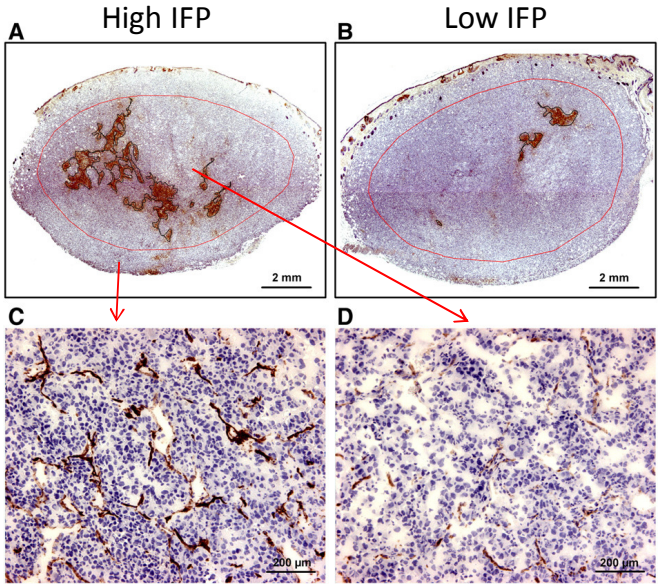
- Largely variable in all histologies
- Cervical cancer has been the most studied human model, followed by head and neck cancer
- Studies in vivo on human melanoma, cervical and breast cancer xenografts

Tumor type	*n*	Mean	Range
Normal skin	5	0.4	−1.0 to 3.0
Normal breast	8	0.0	−0.5 to 3.0
Head and neck carcinomas	27	19.0	1.5 to 79.0
Cervical carcinomas	127	20.5	−2.8 to 94.0
Lung carcinomas	26	9.5	1.0 to 27.0
Metastatic melanomas	26	18.0	0.0 to 60.0
Breast carcinomas	21	23.7	4.0 to 53.0
Brain tumors	28	4.6	−0.5 to 15.0
Rectal carcinoma	8	15.3	12.1 to 15.8
Colorectal liver metastasis	8	21.0	6.0 to 45.0
Lymphomas	7	4.5	1.0 to 12.5
Renal cell carcinoma	1	38.0	–

IFP

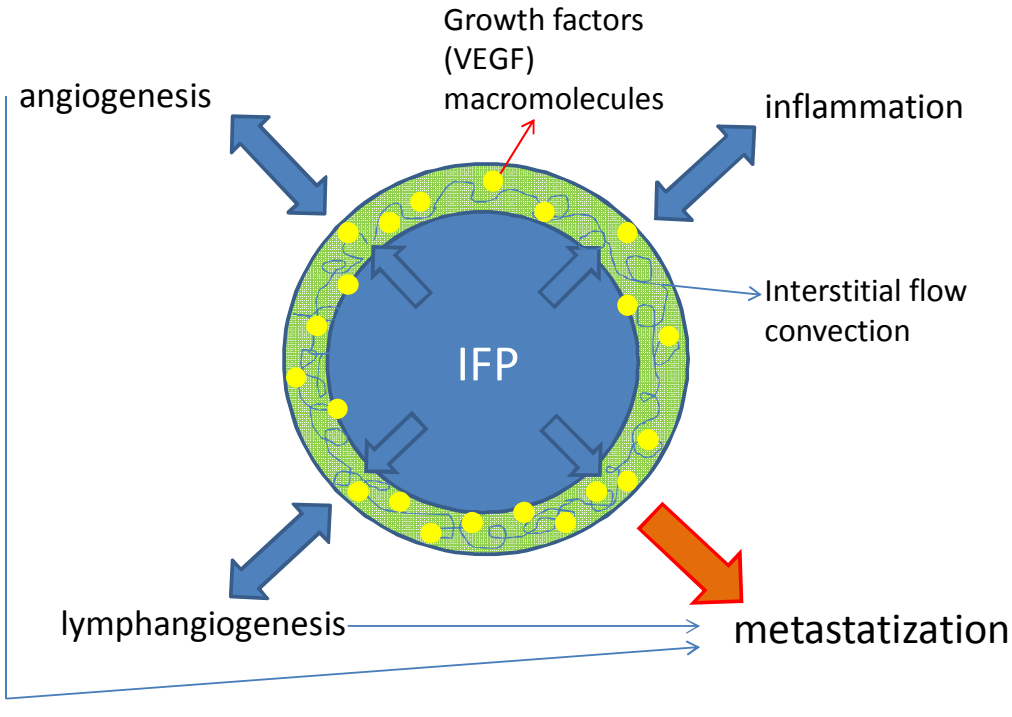
Links with angiogenesis and hypoxia:

- + correlation between IFP and central hypoxic fraction
- + correlation between IFP and peripheral MVD (CD31)
- Critical IFP level: 20 mmHg



Rofstad et al 2014

Peritumor edema

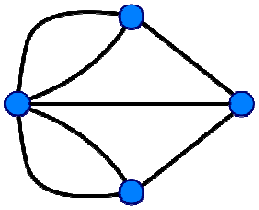


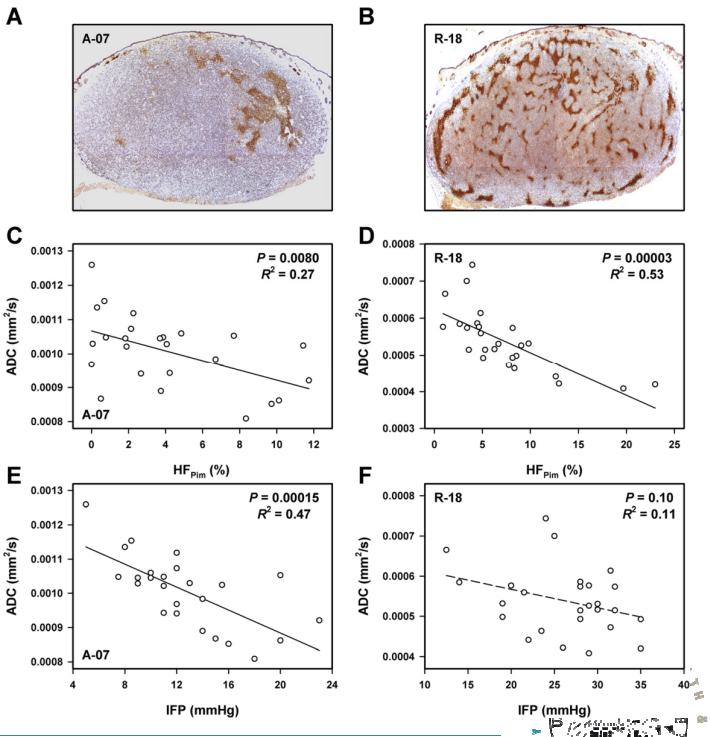
IFP

- Correlation with radiocurability in xenografts: TCD_{50} 20% higher in high IFP (>8 mmHg) [Rofstad et al 2009]
- Correlation with radiocurability in xenografts without hypoxia: TCD_{50} 13% higher in high IFP [Rofstad et al 2010]
- → hypoxia related and non-related effects
- Prognostic factor in cervical cancer [Fyles 2006; Yeo 2009; Hockel 1996 and 1999; Lyng 2000; Knocke 1999]
- Benefit from cisplatin addition to RT in high IFP cervical cancer [Milosevic et al 2014]

DWI→IFP

- ADC inversely correlated with IFP
- ADC inversely correlated with hypoxic fraction
- ADC inversely correlated with tumor cell density
- Cell density correlated with IFP and hypoxia



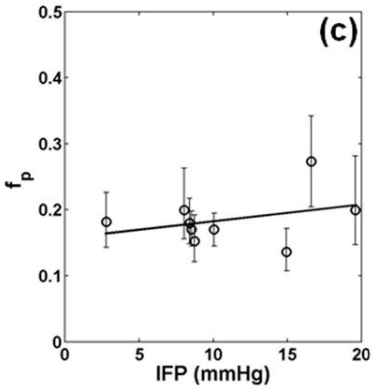


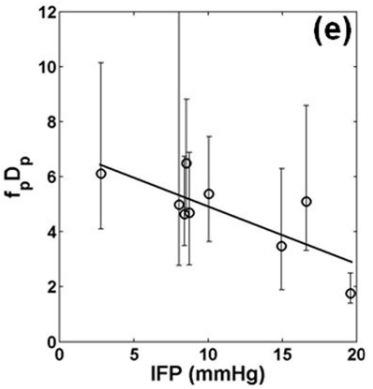
Hompland et al 2014

IVIM DWI→IFP

- IFP correlates with IVIM metrics in a mouse mammary carcinoma model

	All voxels		AVV only	
	r	*p*	*r*	*p*
ADC	0.06	0.87	0.11	0.78
D_t	0.18	0.64	0.30	0.44
f_p	0.35	0.36	0.34	0.37
D_p	0.70*	0.04	0.76*	0.02
$f_p \cdot D_p$	0.70*	0.03	0.77*	0.02





Kim et al 2012

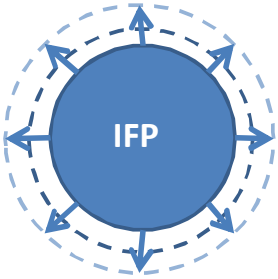
DCE→IFP

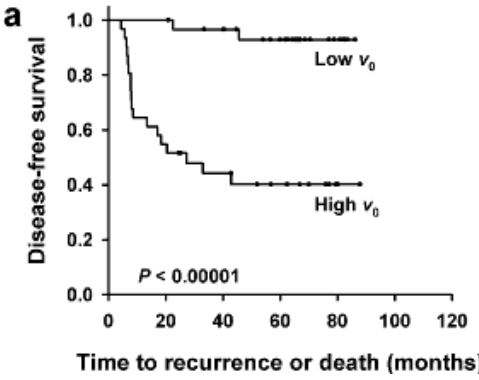
- Microvessel permeability (Ktrans) is inversely correlated to IFP [Hompland et al 2013; Haider et al 2007]
- As suggested also from IVIM studies, high IFP neg affects perfusion and oxygenation of tumor
- Mathematical models suggest possible role of antiangiogenetic drugs in normalizing interstitial hypertension [Jain et al 2007] → association with radiotherapy?

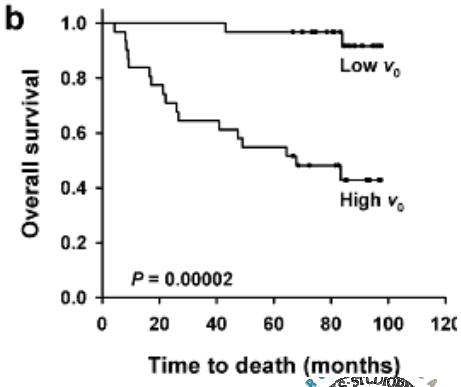
Kim et al 2012

DCE→peritumor edema

- Peritumoral interstitial fluid flow velocity measured by DCE-MRI predicts survival in cervical carcinoma (62 pts)
- Velocity of outward expansion of peritumoral enhancement

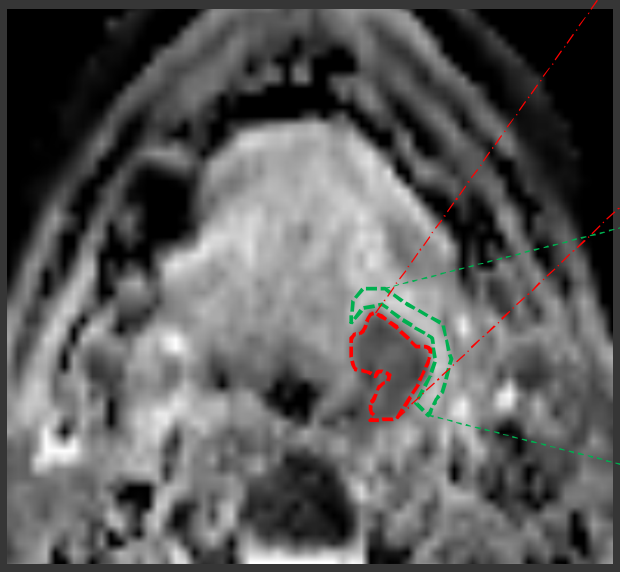


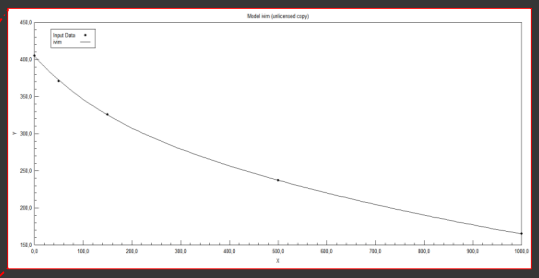


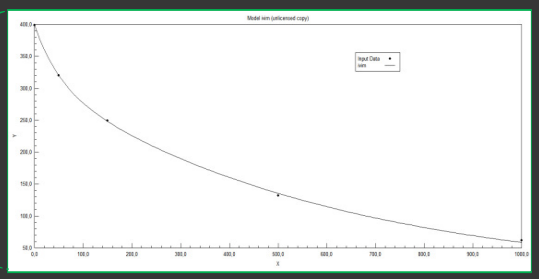


Hompland et al 2013

IVIM→peritumor edema

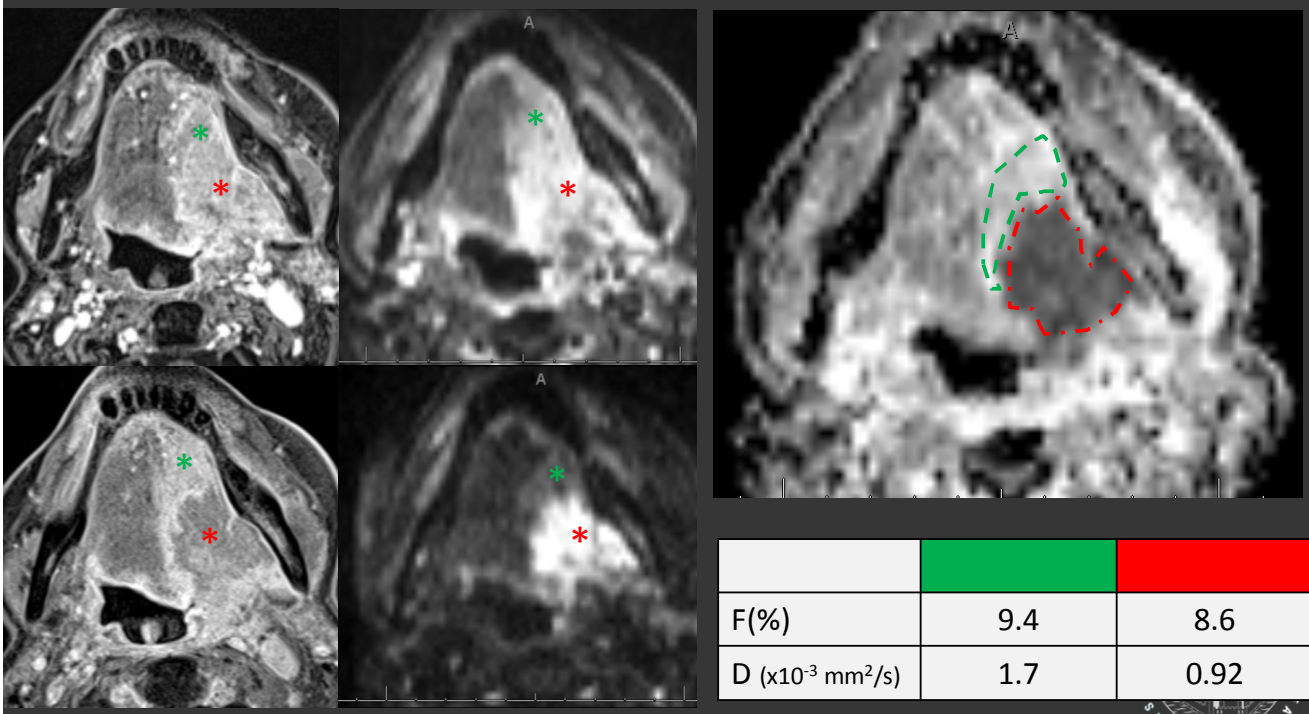






F(%)	21	9
D (x10^{-3} mm^2/s)	1.67	0.82

IVIM→peritumor edema

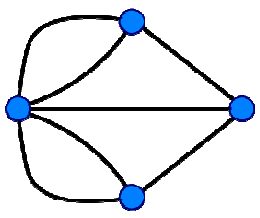


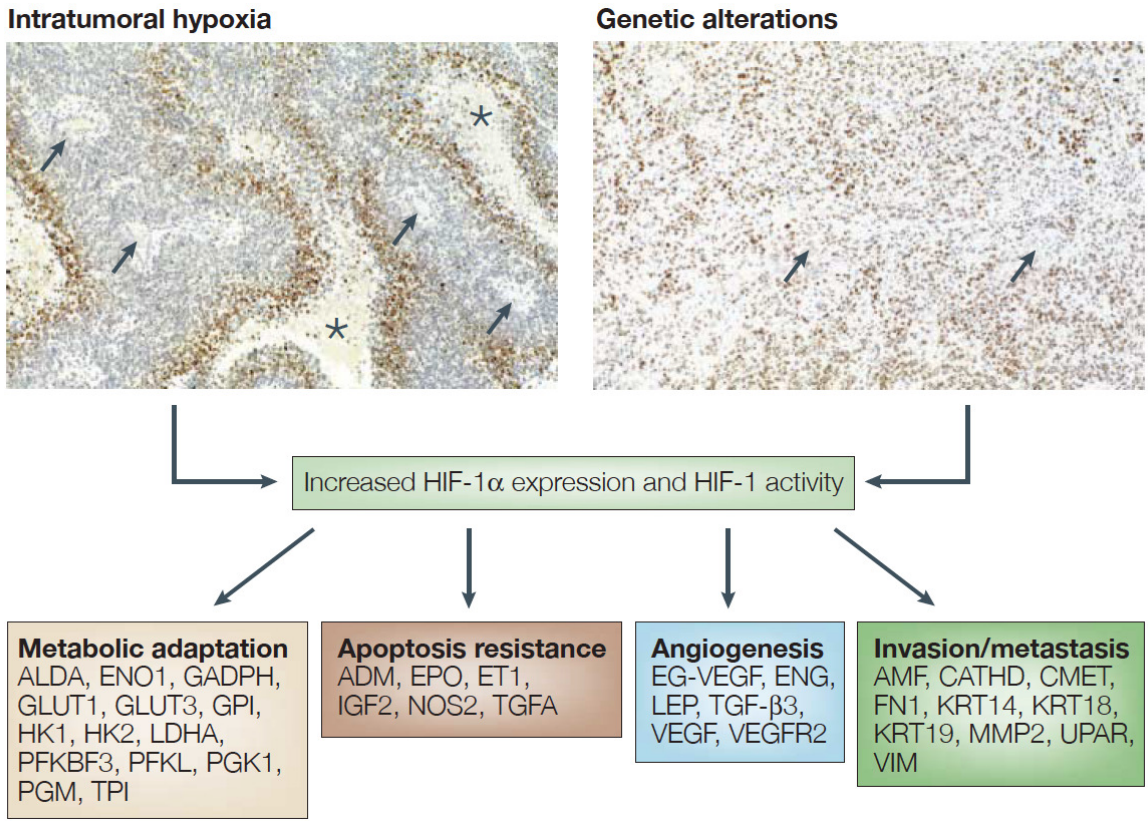
F(%)	9.4	8.6
D (x10^{-3} mm^2/s)	1.7	0.92

tumor hypoxia

Direct

- acute: perfusion-related
- chronic: diffusion-related, increased diffusion distance more than 70-100 µm
- Promotes angiogenesis, adaptation and immortalization via HIF-1





cyclic hypoxia (pre-clinical)

- cyclic acute: cyclic fluctuations in tumor oxygenations given by acute hypoxic followed by reoxygenation phases;
- hypoxic phases: HIF-1α accumulation in endothelial cells;
- reoxygenation phases: signalling cascade leading to phenotypic changes, genome instability.

- incremented angiogenesis
- increased metastatisation
- immortalization

- increment of cancer stem cells (CSC) population

acute cyclic hypoxia (in vivo)

- *In vivo,* acute cyclic but not chronic hypoxia induced increased metastatisation [Cairns et al 2001]
- acute cyclic hypoxia enhances angiogenesis [Gaustad et al 2013]
- tumors exposed to acute hypoxia are more radioresistant than chronicall hypoxic tumors [Denekamp et al 1999]
- chronic and cycling hypoxia differently affect different hystotypes [Ellingsen et al 2012]

need for techniques capable to measure hypoxia and separate acute cyclic from chronic hypoxia

hunting for hypoxia

- oxygen probes (computerized pO2 histography) demonstrated that hypoxia is not dependent on size, stage, histology and grade in uterine cervix cancer. [Vaupel et al 2001]
- hypoxic areas are heterogeneously distributed in the tumor [Vaupel et al 2004].

whole tumor individual assessment (imaging)

Optimal (imaging?) technique

- **Quantitative:** effectiveness of different therapies becomes impaired at different pO2 levels [Hockel et al 2001], 0-15 mmHg level seems to be critical.
- **Sensitive** to small pO2 changes.
- **Specific:** Direct on hypoxia or on specific hypoxia effects.
- Able to separate **chronic, acute/cycling** hypoxia and anoxic necrosis.
- Able to image the **whole tumor** (not only superficial tumors).

possible strategies

- assessment of tumour oxygenation **Direct**
  - pO2 measurement
  - oxygenation-dependent biological pathways
- assessment of hypoxia phenotypes **Surrogate**
  - perfusion assessment

PET world

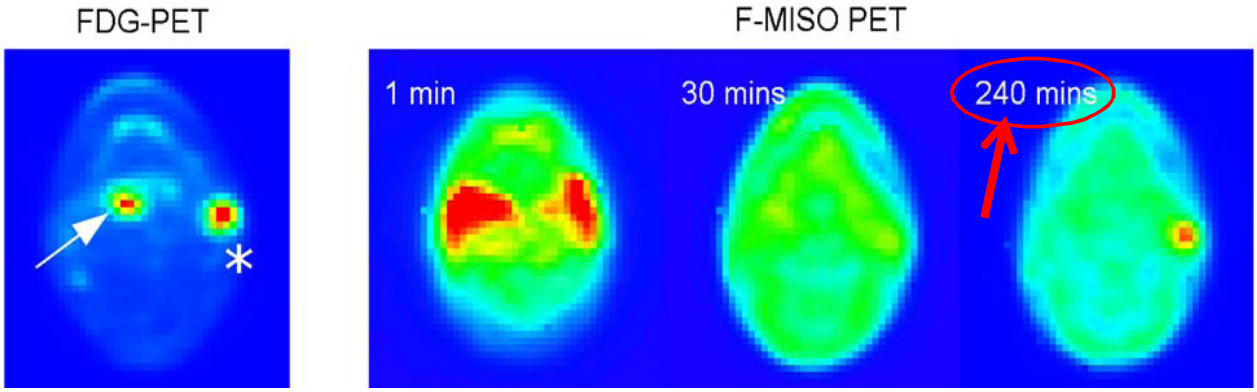
Direct

- Nitroimidazole based: **^{18}FMISO** (FDA approved), ^{18}FAZA, ^{18}FETA etc.
- Etanidazole based: EF3-EF5
- **^{64}Cu ATSM** (FDA approved): higher signal to background ratio, 12h half-live
- **HX4: most promising**
- FDG: non specific.
  FDG uptake does not correlate with hypoxia specific stainings/tracers uptake.

F-18–fluoromisonidazole (F-MISO PET)

- most commonly used radiotracer in hypoxia imaging;
- misonidazole passively diffuses into the cells:
  - in the presence of oxygen, the last reaction is reversible and the molecule can leave the cell,
  - in absence of oxygen, misonidazole is reduced and remains trapped in the cell.
- also an efficient hypoxic radiosensitizer;

^{18}F MISO PET



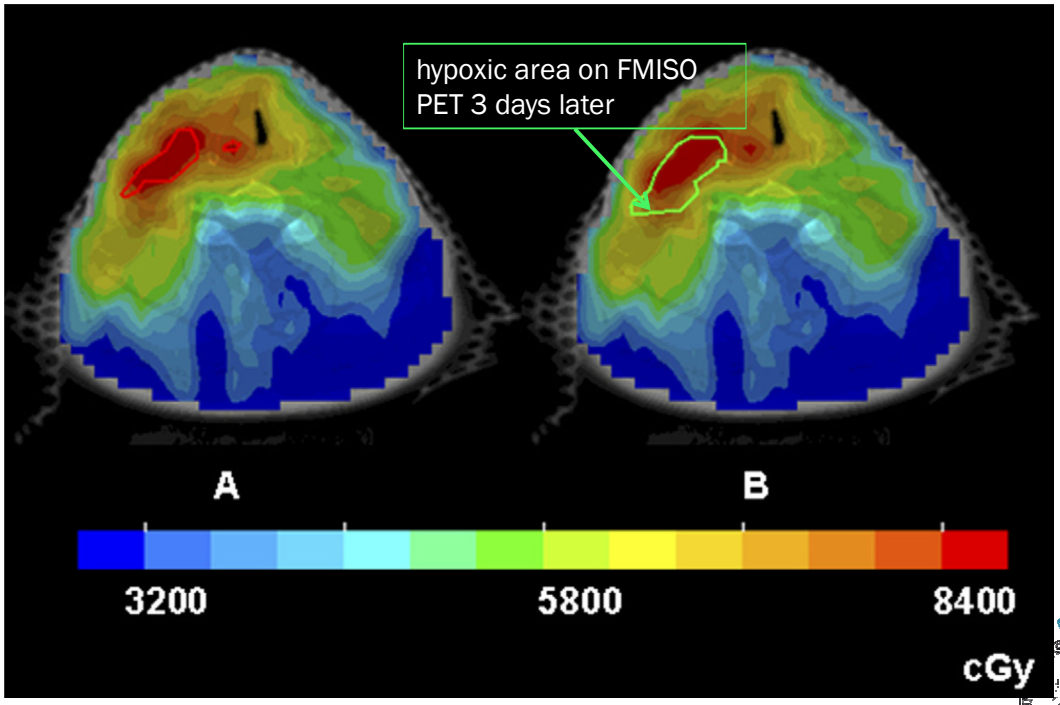
- No differentiation between chronic and acute cycling hypoxia
- Seems to be affected by both [Monnich et al 2012]

Hypoxia/metabolism geographical mismatch

- HX4 PET on 20 head and neck cancer patients
- 13/20 hypoxic
- Hypoxic usually smaller than metabolic subvolumes (51%±26%)
- In 9/13 25%±21% of hypoxic subvolume was outside metabolic subvolume
- → FDG PET cannot be used as surrogate of hypoxia imaging
- Similar results on NSCLC [Zegers et a 2014]

Zegers et al 2015

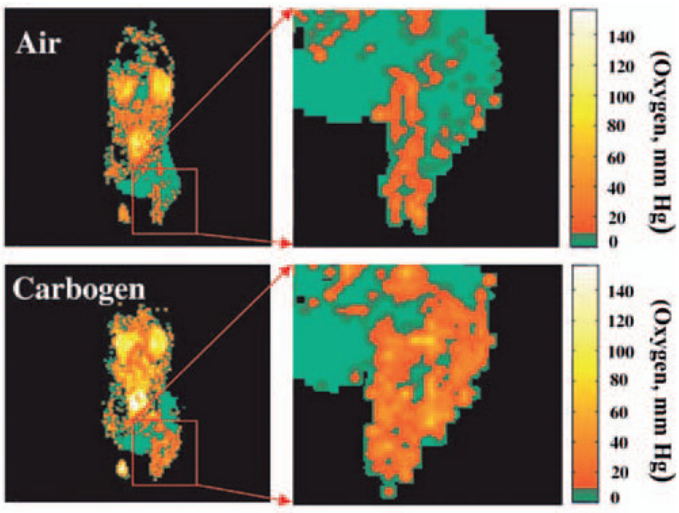
Hypoxia (t)



Lin et al 2008

MR world Direct

- Electron paramagnetic resonance imaging (EPRI) and Overhauser MRI (OMRI): measures redox status of injected nitroxides or trytil radical, determined by tissue molecular oxygen
- High temporal resolution allows detection of cyclic hypoxia



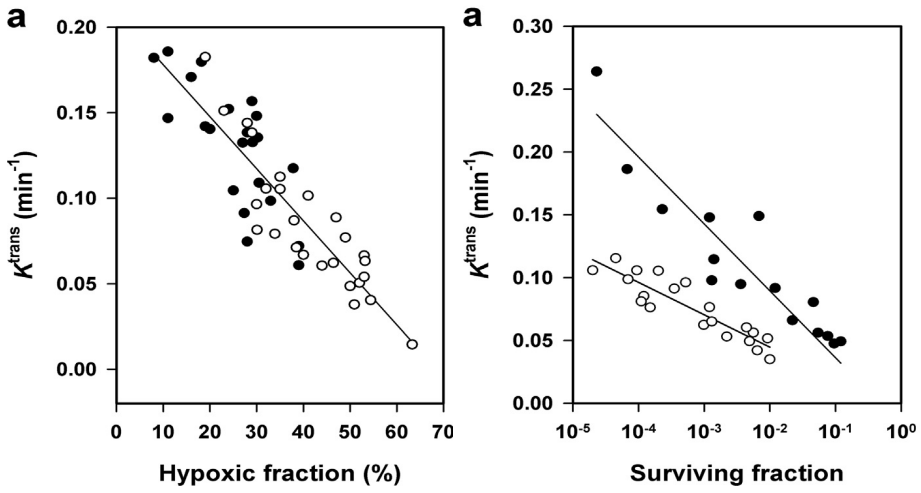
Krishna et al 2013

MRI world Surrogate

- BOLD MRI (usable in clinics):
  - does not measure pO2 (no linear relation), but deoxyhemoglobin concentration
  - flow-dependent → sensitive to acute hypoxia
  - flow-dependent → influenced by regional blood flow
  - relatively insensitive to chronic hypoxia (occurring in non-flowing blood regions)
  - Poor quantitative correlation with pimonidazole staining

DCE MRI

- Correlatin with hypoxic fraction and radioresponsiveness in cervical carcinoma xenografts



Ellingsen et al 2014

DCE MRI Surrogate

- Data confirmed in vivo on xenografts by several studies
- Except: poor correlation in rectal cancer [Atkin et al 2006; Kim et al 2013]
- Correlation between DCE MRI and hypoxia markers in humans is emerging in:
  - cervical cancer [Halle et al 2012]
  - prostate cancer [Borren et al 2013]
  - gliomas [Jensen et al 2014]

Hypoxia imaging

- Prediction and prognostic risk stratification:
  - FMISO: Sato et al 2014 (H&N), Trinkaus et al 2014 (H&N), Zips et al 2013 (H&N), Hugonnet et al 2011 (kidney) Rischin et al 2006 (H&N), Eschmann et al 2005 (NSCLC)
  - DCE MRI: Jensen et al (gliomas), Halle et al 2012 (cervix), other studies but without hypoxia specific evaluation
- Promising results for objective response and progression-free survival, not for overall survival

Angiogenesis

- MVD (CD31) the most used marker
- Radiosensitivity: high MVD associated with higher radiosensitivity in early laryngeal cancer [Kamijo et al 2000] and metastatic cervical lymph nodes from HNSCC [Ito et al 2011]

- Outcome (MVD, multimodal treatment): no correlation with outcome in HNSCC [Calvin et al 2007; Foote et al 2005], poor prognostic factor in breast cancer [meta-analysis, Uzzan et al 2004; Gasparini et al 2001], renal cancer [meta-analysis, Cheng et al 2014; Zhang et al 2014] and CRC [Des Guetz et al 2006]

Angiogenesis

- MVD seems to be positively correlated with radiosensitivity and poor prognosis!!!

REVIEW

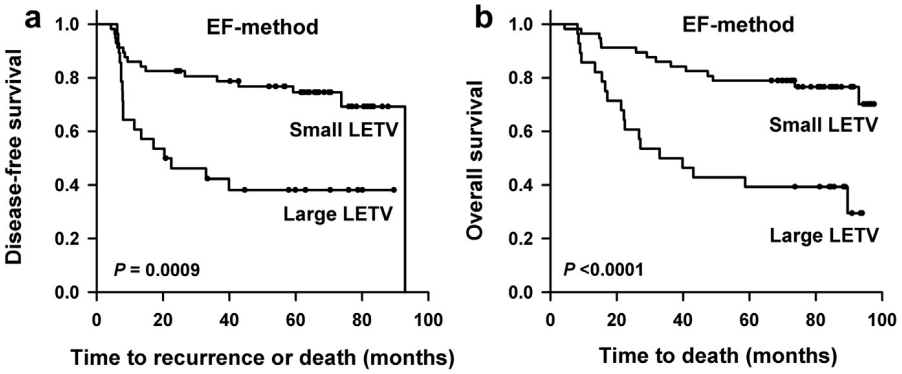
Clinical Application of Antiangiogenic Therapy: Microvessel Density, What It Does and Doesn't Tell Us

Lynn Hlatky, Philip Hahnfeldt, Judah Folkman

- MVD describes number of vessels per hotspot but not vessel funcion neither angiogenetic activity
- Furthermore, microvascular heterogeneity must be taken into account

DCE-MRI

- Non pharmakokinetic quantitative analysis (highly enhancing pixel fraction at 60 sec), 85 pts, advanced cervical cancer

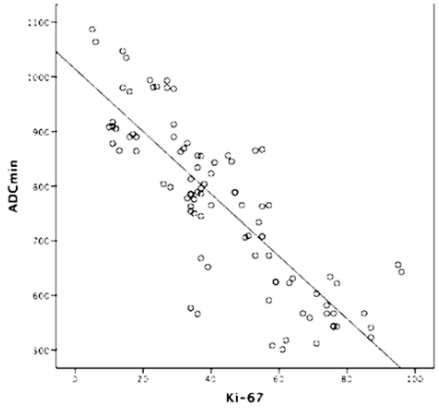


- Similar results (K_{trans}) confirmed in other studies [Mayr et al 2011]
- DCE-MRI provides information about vascular function, correlating with angiogenetic activity

Lund et al 2015

DWI→Cell proliferation

- 93 NSCLC, pre treatment DWI, ADC min

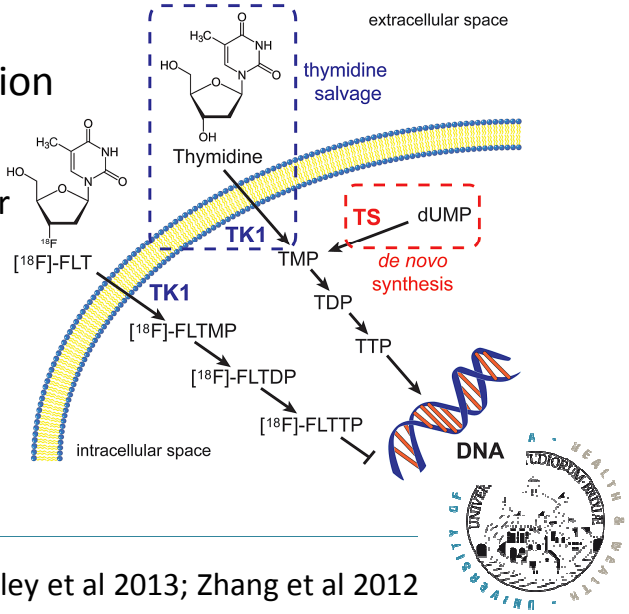


- (Known correlation with cell density)
- Also observed in breast cancer [Molinari et al 2015]

Karaman et al 2015

Direct FLT PET→proliferation

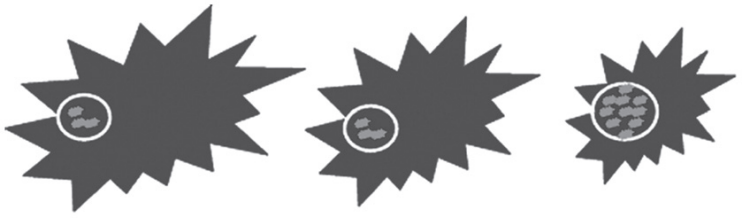
- Thymidine salvage way (DNA precursors supply)
- Dependent on TK1 activity (late G1-S phase)
- Non linear and heterogeneous relationship with cell proliferation
- Correlation demonstrated in: lung cancer, B-lymphoma, skin cancer but not in: CRC, neuroblastoma and several xenograft models
- Prognostic prediction in high grade glioma [Idema et al 2012] et lymphoma [Hermann et al 2011]



McKnley et al 2013; Zhang et al 2012

FLT-PET→repopulation

- Yue et al (2010) demonstrated increase FLT uptake in 2 pts after RT interruption
- Everitt et al (2009) observed a 'flare' of 18F-FLT uptake in NSCLC following only 2 Gy irradiation
- Fatema et al (2013) demonstrated gradual increase of FLT uptake in HNSCC xenografts since 6 hr after treatment



Necrosis/apoptosis

- Possible biomarker of treatment efficacy
- Early assessment with DWI during treatment.
- Rationale for DWI is increase water diffusivity due to cell membrane rupture
- Increase in ADC during treatment is sign of response
- ADC changes <10-15% are *technically* non significant because of low test re-test repeatibility

DWI during-Chemo-RT & post-ChemoRT

early PET CT
SE 83% SP 54%

reference	year	type	pts	T	N	time
Vandecaveye	2010	pro	30	Δ ADC> 14% SE 88% SP 91%	Δ ADC> 14,6% SE 80% SP 89%	2 weeks
				Δ ADC> 25% SE 100% SP 91%	Δ ADC> 19% SE 80% SP 96%	4 weeks
King	2		20 (50)	ADC < 1,43 ×10^{-3} mm^2/s SE 45% SP 100%		6 weeks post treatment end
Kim	2009	pro	33		ADC < 1.11 SE 65% SP 86%	pre Tx
Vandecaveye	2007	pro	26	ADC > 1,3 ×10-3 mm2/s SE 94% SP 95%		post treatment end
Razek	2006	pro	32	ADC < 1,3 ×10-3 mm2/s SE 84% SP 90%		post treatment end
Kato	2009	retro	28	ADC correlates with regression rate (r -0.37)		pre treatment

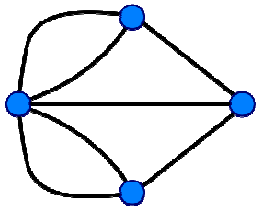
Δ > 35%

Δ > 11%

6/30 papers in last 5years PubMed (DWI OR diffusion MR)

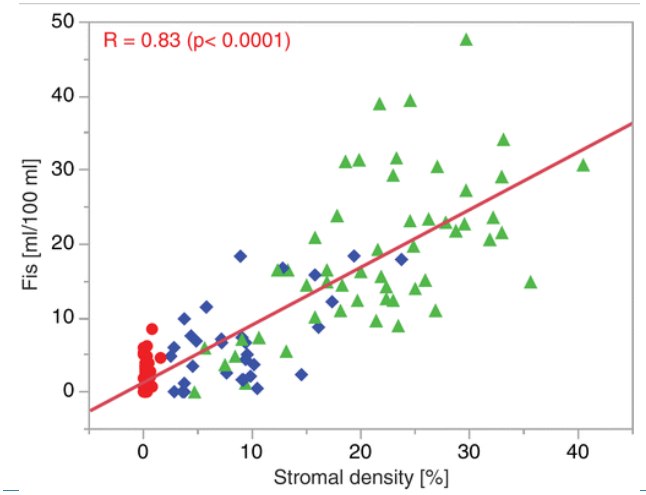
Tumor stroma

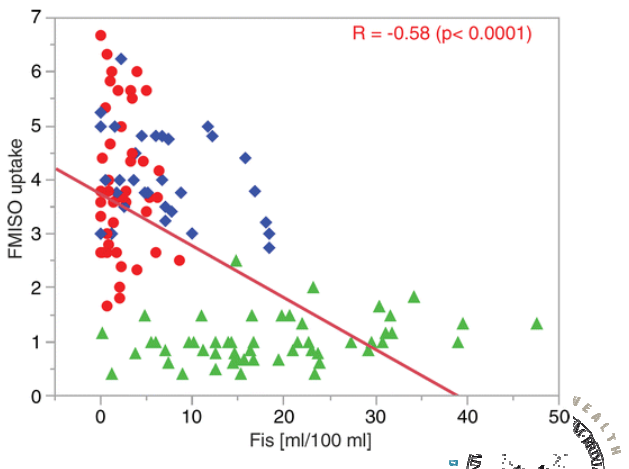
- Strong prognostic variable in lung adenocarcinoma [Maeshima 2002], gastric cancer [Wu 2013], triple-negative breast cancer [Moorman 2012]
- Complex interactions with angiogenesis, hypoxia, immunity
- Complex and debated role in radiosensitivity [Ogawa et al 2007]



DCE CT and tumor stroma

- Fis describes contrast accumulation in the interstitium
- Strong dependence on tumor histotype

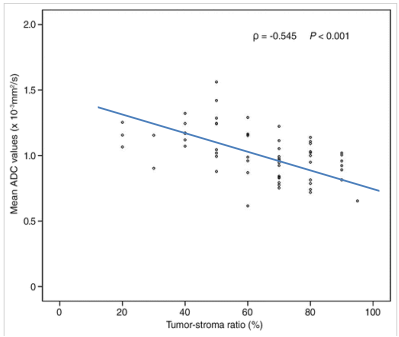




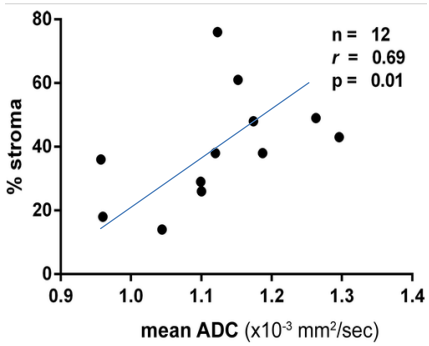
Koyasu et al 2015

DWI and tumor stroma

- Heterogeneous results
- Heterogeneous histology: collagen-dominant, fibroblasts-dominant, lymphocyte-dominant
- Possible role for direct targeting with PET [Blykers 2015]



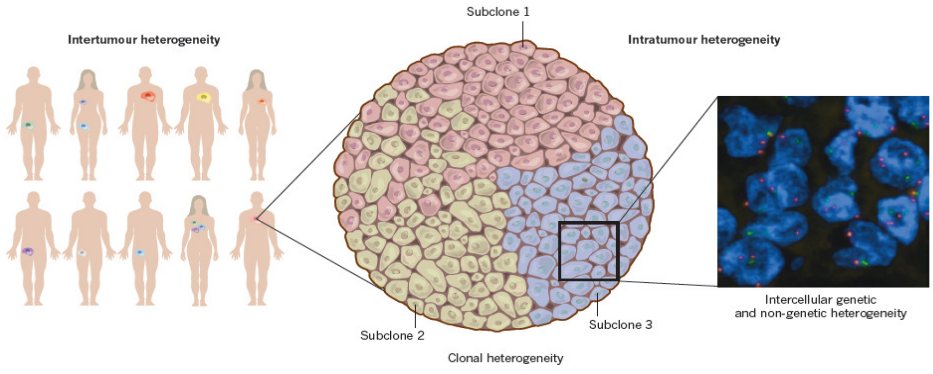
Breast ER+ cancer [Ko et al 2014]



Laryngeal cancer [Driessen 2014]

Tumor heterogeneity

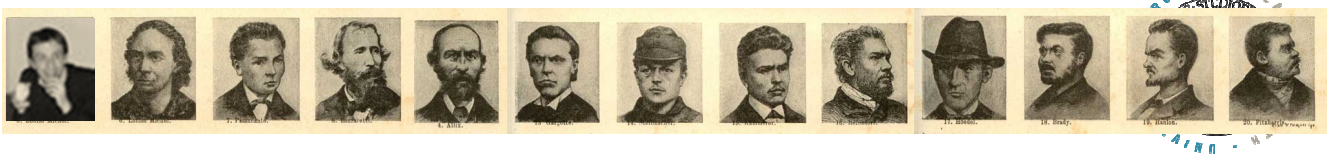
- Inter-tumor heterogeneity
- Intra-tumor heterogeneity (multiclonality, stochastic genetic or epigenetic events, microenvironmental pressure fluctuations)
- Heterogeneity among tumor and its metastases



The *Lombrosian* hypothesis

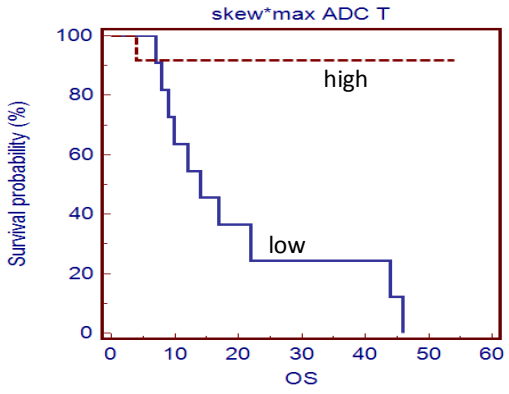
- Imaging depicts tumor heterogeneity at phenotypic level
- *Phenotypic tumor heterogeneity reflects genetic, epigenetic, microenvironmental heterogeneity*
- Genetic, epigenetic, microenvironmental heterogeneity influences treatment response

→ imaging can predict treatment response



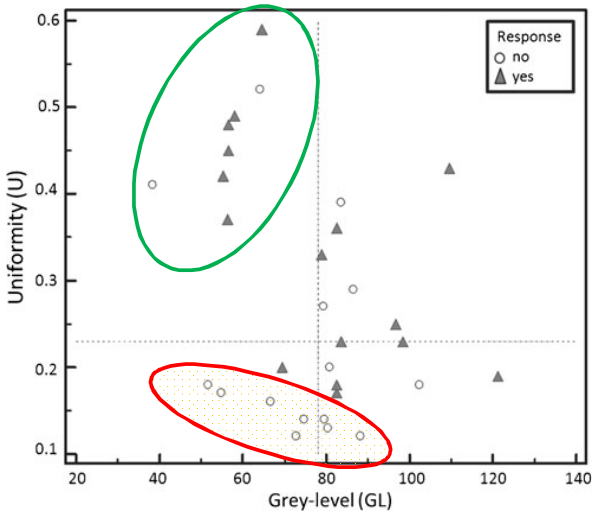
DWI heterogeneity in OPSCC

- 27 pts with advanced oropharyngeal cancer treated with CHT-RT
- Histogram analysis on DWI before treatment
- ADC skewness and max on T correlated with prognosis (skew*max had RR=15.45 for OS, p<0.0001)
- Critical issues:
  - repeatibility
  - tumor segmentation



CT heterogeneity in NSCLC

- Retrospective study on 53 pts with advanced NSCLC
- Texture analysis on *pre-treatment* contrast-enhanced CT
- CT density and Uniformity predicted objective response to CHT
- Possible patient stratification



Ravanelli et al 2013

filtration-histogram approach (literature overview)

- Colorectal cancer: hepatic CT on portal phase-baseline [Ganeshan et al 2007]
- Breast cancer: MR-early assessment [Parikh et al 2014]
- Oesophageal cancer: unhenanced CT-baseline [Ganeshan et al 2012]
- NSCLC: unhenanced CT-baseline [Ganeshan et al 2012];
  NSCLC: contrast-enhanced CT-baseline [Ravanelli et al 2013]
- Renal cancer: contrast enhanced CT-early assessment (antiangiogenetic drugs) [Goh et al 2011]
- H&N: contrast enhanced CT-baseline [Zhang et al 2013]

CT heterogeneity in CRC liver mets

- 23 pts CHT + bevacizumab, 20 pts CHT
- Texture analysis on *pre-treatment* contrast-enhanced CT
- Correlation with objective response, PFS and OS
- In pts treated with bevacizumab, high uniformity correlated with poor response rate and prognosis (OR=20 for objective response RR=5.1 for PFS, 6.7 for OS)
- No correlation in pts treated by CHT alone

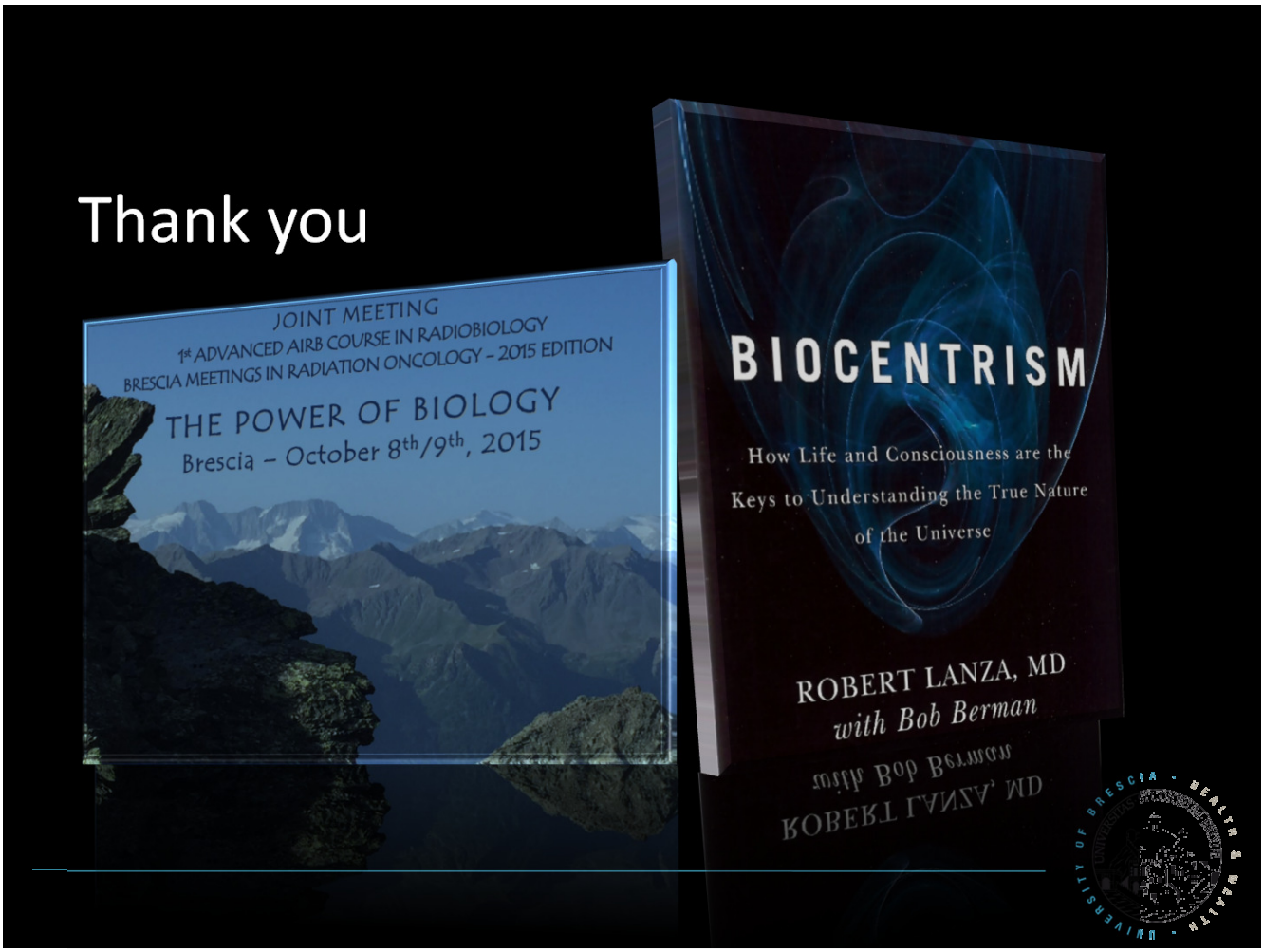
→ Selection of pts who would benefit from addition of bevacizumab to CHT avoiding overtreatment

Ravanelli et al ECR 2015

Final considerations

- Imaging insights in tumor biology
- Imaging reflects on large mm-scale the tumor phenotype resulting from complex interactions of several micro/nano scale factors
- Evidence on xenografts are quite strong but strongly dependent on histotype
- Standardization of techniques and large studies are needed in humans
- What does it mean? → Does it works?

Thank you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