UCSF Helen Diller Family Comprehensive Cancer Center

Drug Measurements at the Pharmacological Target Site for Individualized Pediatric Cancer Treatment

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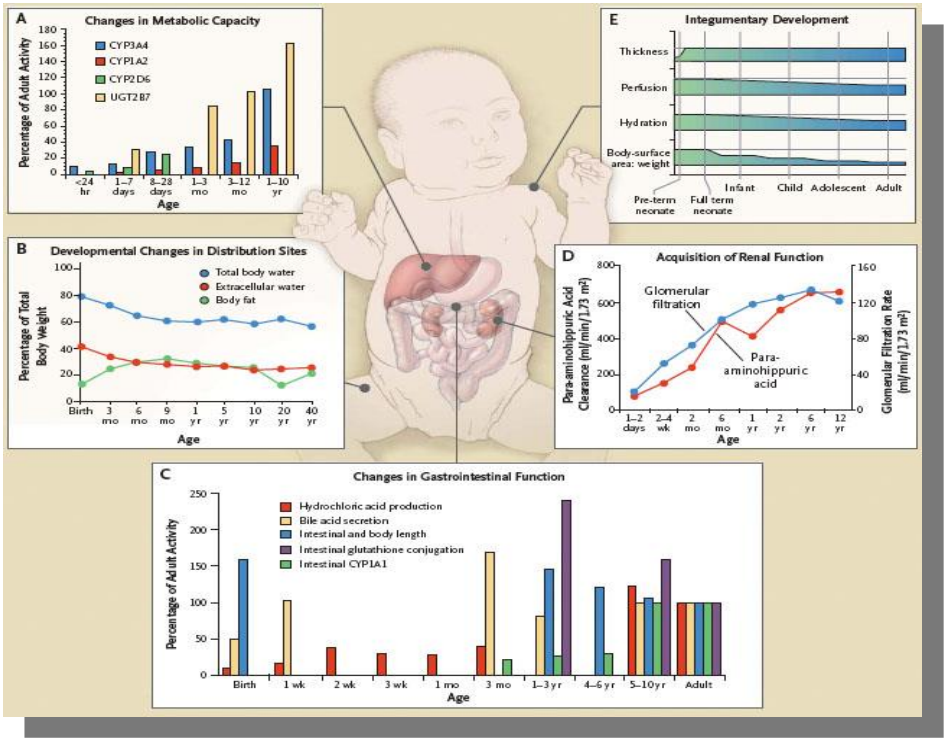
Children are not small adults!



- Different **blood exposures** adults and children
- Only some trials account for different plasma pharmacokinetics in age-categories

Kearns, G.L. *et al.* NEJM, 349(12): 157-67, 2003

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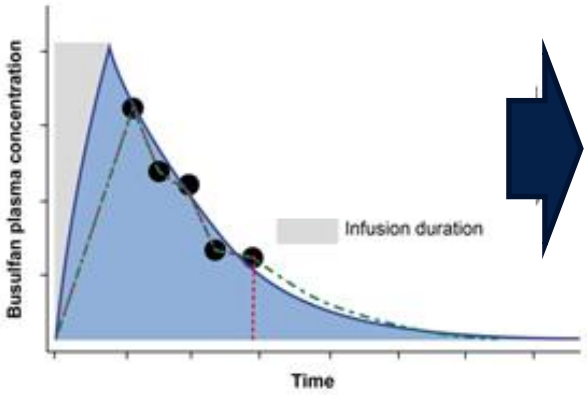
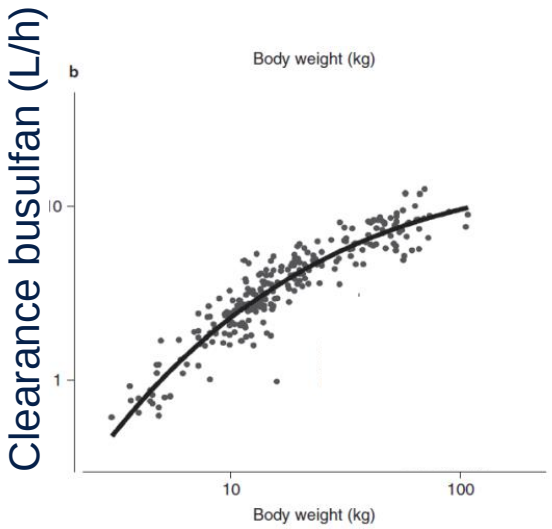
Dosing schedule accounting for age-related pharmacokinetics, example busulfan

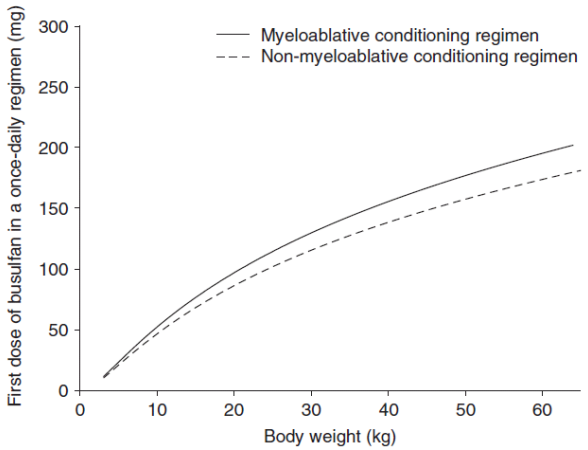
Estimate exposure



Pharmacokinetics



Individualized dosing



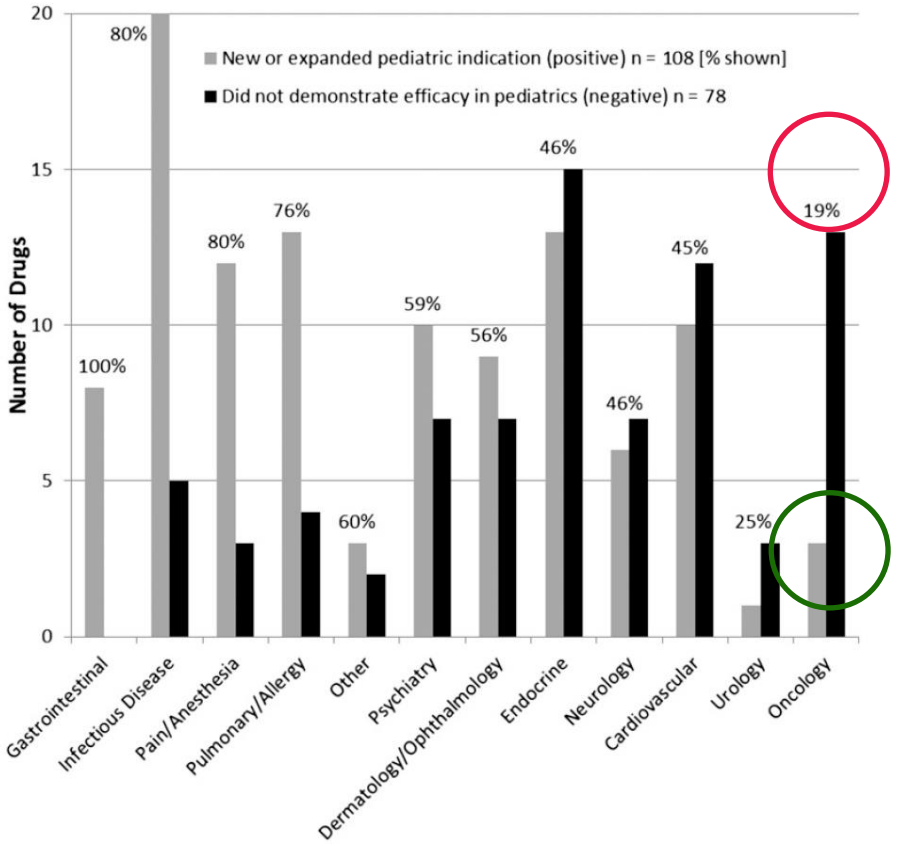
- Exposure: Area Under the Concentration-time curve (**AUC**) = dose*bioavailibility/Clearance
- Dose optimized= AUC_{target}*$Dose_i$ /AUC_i

Other examples of PK-PD based individualized pediatric doseing

- Midazolam in based on PK difference in children at intensive care unit (PICU) and non-PICU[1]
- Warfarin dosing in children based on exposure-INR[2]
- Vigabatrin, assesment of dose-response children and adults[3]
- Esomeprazole PK in children and adults[3]
- Adalimumab, exposure-response, PK, efficacy and safety assessed in pediatric crohn's disease.[3]

1. Ince *et al.* TDM 34; 381-389, 2012
2. Hamberg *et al.* BMC MIDM 15 (7): 1-9, 2015
3. Mehrotra *et al.* DMD #69559, 2016

Demonstrating Efficacy For Oncologic Agents Remains a Challenge



1. Pemetrexed
2. Erlotinib
3. Capecitabine

Even when correctly accounting for plasma exposures, pediatric oncology trials fail

Wharton *et al.* Pediatrics 134 (2) e512-e518, 2015

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Beyond genomics, challenges in pediatric drug development

	Obstacle	Observed in	Pediatric predictions addressed in this presentation
1	Dose/m2 or /kg ≠ uniform exposure	Children of different ages	Age and drug specific dosing needed
2	Concentration plasma ≠ tumor	Adults	Higher variability
3	Variability in drug penetrance per tumor type	Adults	Translation difficult
4	Heterogeneity in spatial + temporal drug distribution	Adults and children	Similar in adults and children
5	Variability in intracellular uptake and transformation	Adults and children	Developmental variability
6	Implement target site-outcome association	Adults	Larger barriers

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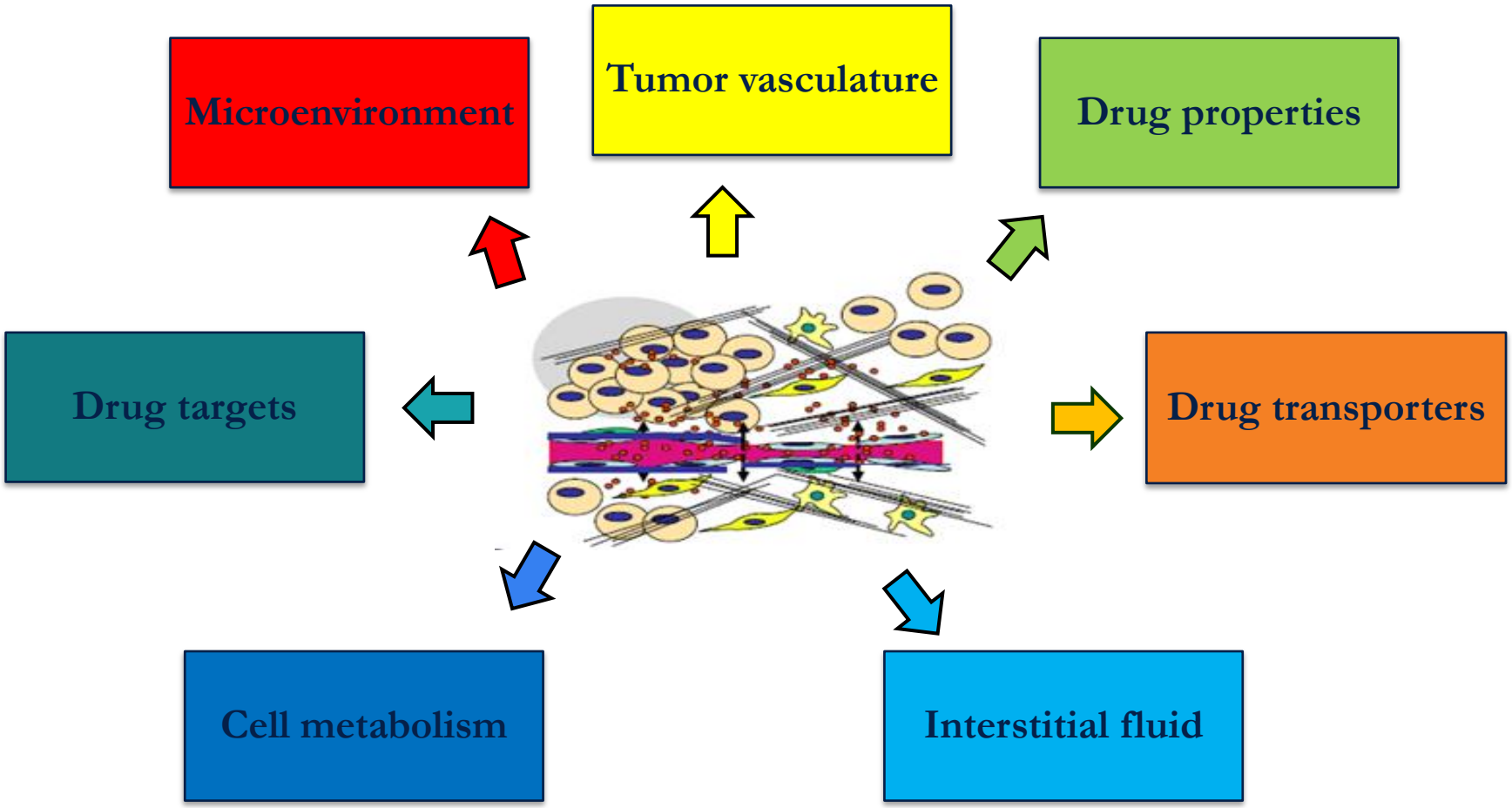
Why is drug distribution in tumor tissue heterogeneous?



Adapted from Nerini *et al.* CPT 96 (2): 224-238,2014

Why do we care to measure drug at the site of action?

- Children are often under or over-treated depending on age and disease.
  - Toxicity with higher exposures can have life-long side effects
  - Sub-therapeutic therapy often results in failure of clinical trials
- In basket-trials multiple diseases are grouped together
- Using drug measurements at the pharmacological target site can be used to
  - Inform dose selection and pharmacodynamic endpoints in early phase pediatric clinical trials
  - Understand difference in outcome between diseases
  - Individualize pediatric cancer treatments to limit "non-responders"
  - Improve efficacy with drug delivery to tumors

How do we measure drug at the site of action?

1. Dose/m2 or /kg ≠ uniform exposure
2. Concentration plasma ≠ tumor
3. Variability in drug penetrance per tumor type
4. Heterogeneity in spatial + temporal drug distribution
5. Variability in intracellular uptake and transformation
6. Implement target site-outcome association

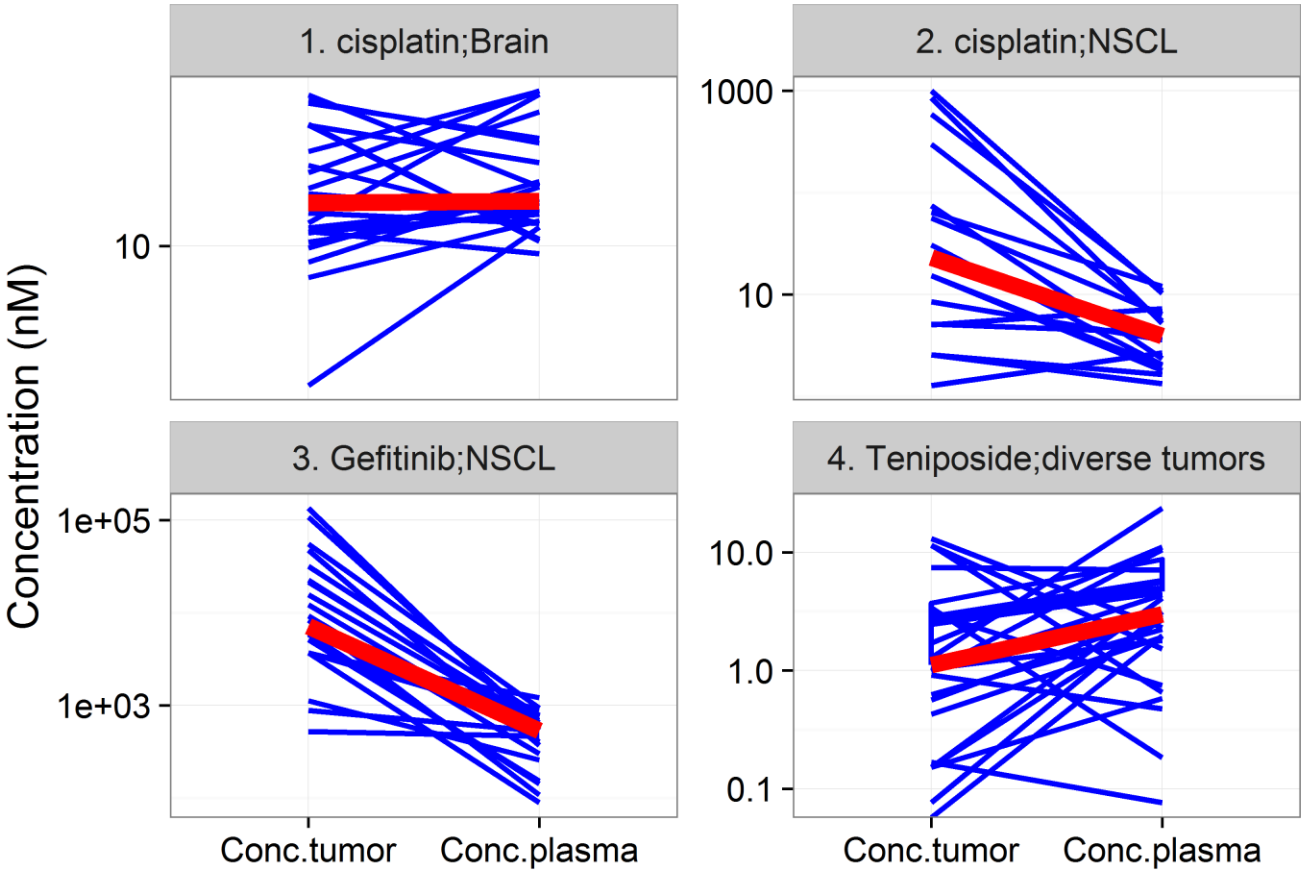
Examples

LC-MS

MALDI, imaging

Extractions or fluorescence

Poor correlation be plasma drug levels and the site of action



Adapted from

1. Nakagawa *et al.* JNO 16:61-67, 1993
2. Pujol *et al.* CCP 27:72-75, 1999
3. Haura *et al.* JTO 5:1806-1814, 2010
4. Stewart *et al.* JNO 2; 315-324, 1984

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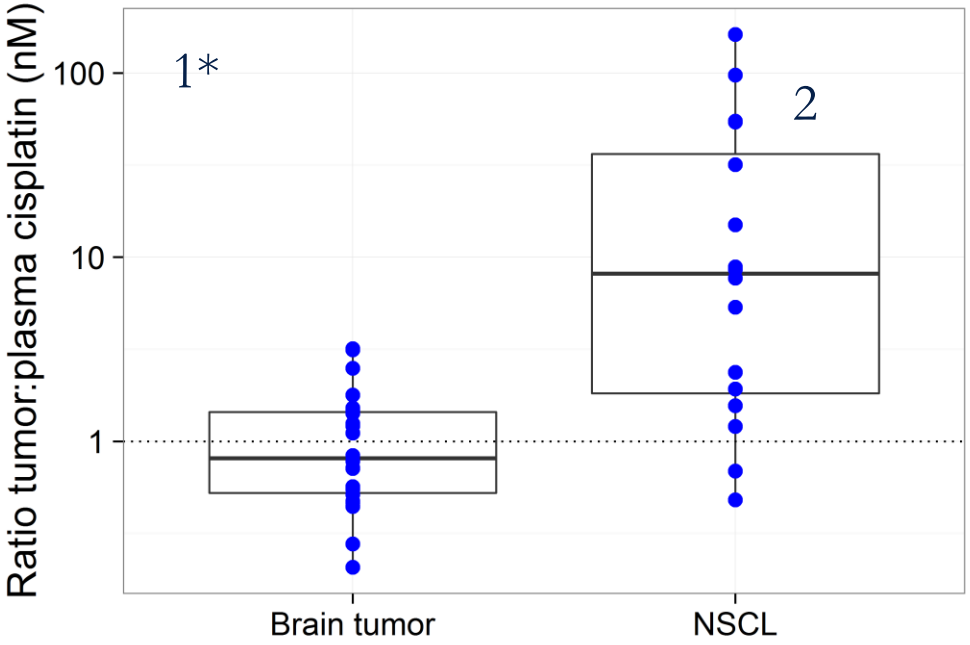
Drug penetration may differ between tumor types

Large uptake in NSCL
*Study differences:

Sampling time differs slightly
Measurment standardization differs: Ref 1: tumor wet weight, Ref 2: tumor dry weight



1. Nakagawa *et al.* JNO 16:61-67, 1993
2. Pujol *et al.* CCP 27:72-75, 1999

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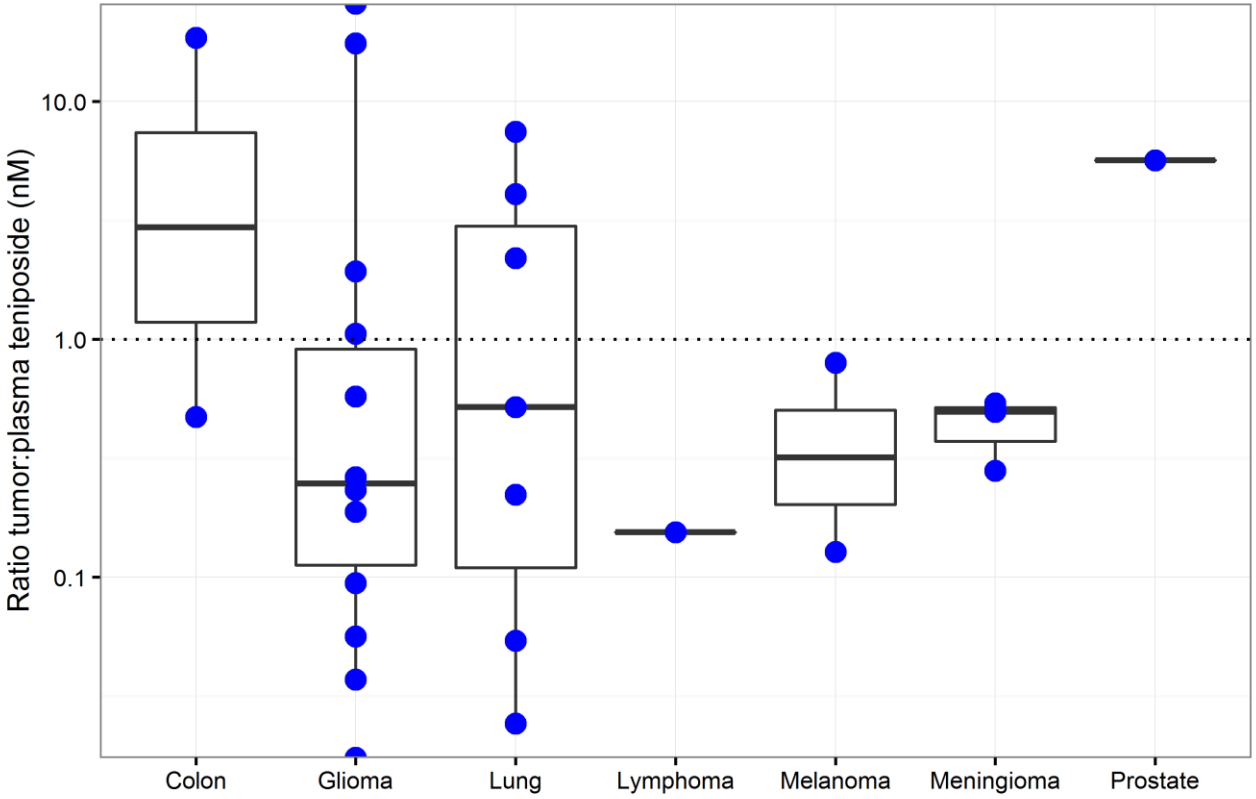
Teniposide penetration may differ between tumor types



Haura *et al.* JTO 5:1806-1814, 2010

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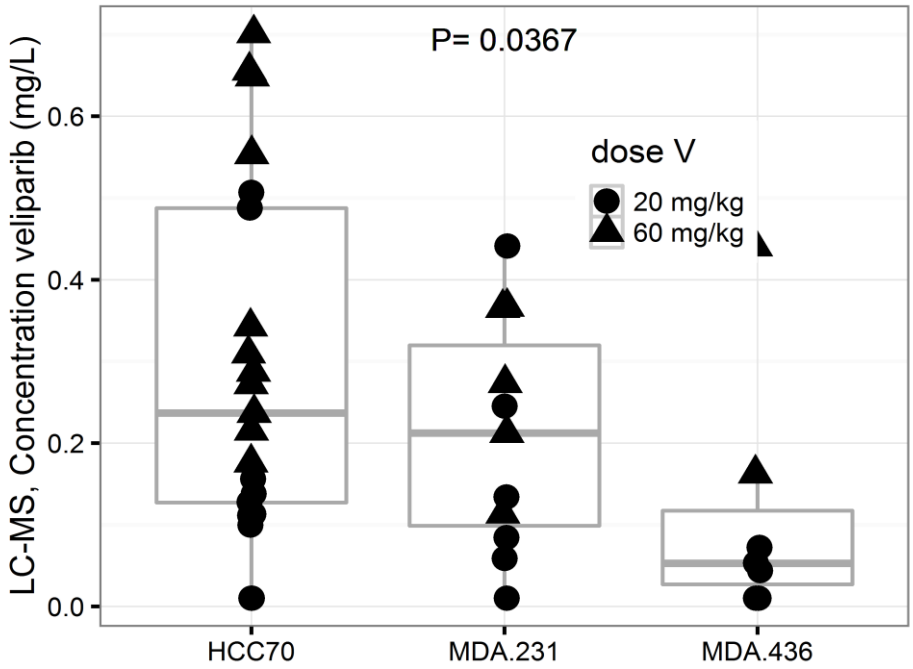
Triple negative breast cancer xenograft study
suggest that veliparib/carboplatin tumor penetration varies



Veliparib concentration measured by LC-MS, 2h after administration 20 mg/kg or 60mg/kg dose (at Steady State)

Bartelink IH *et al.* CCR 2016 (submitted)

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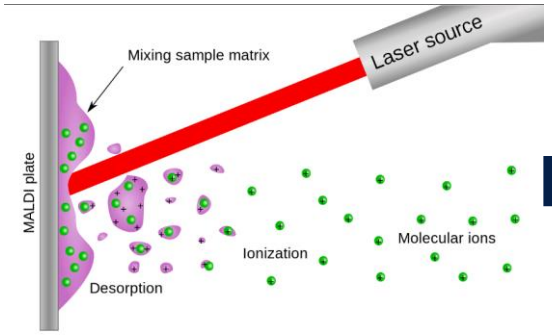
MALDI-MSI

Matrix-assisted laser desorption/ionization mass spectrometry imaging for measuring heterogeneity

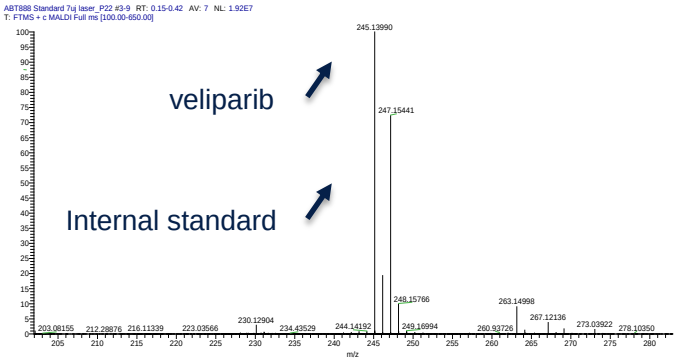
Tissue section

Matrix application

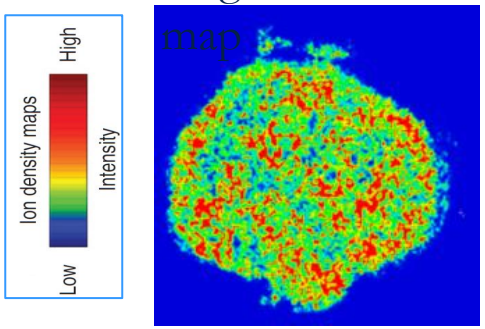
MALDI-MSI measurement



Data analysis



Single ion intensity map



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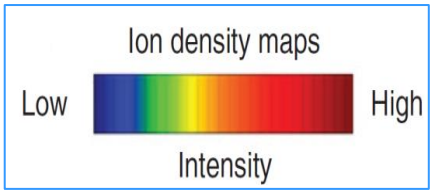
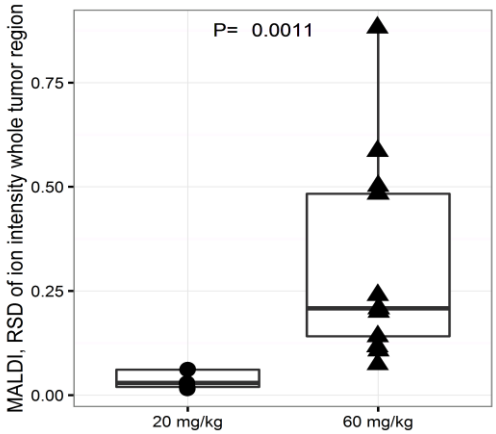
MALDI-MSI shows heterogeneous veliparib penetration

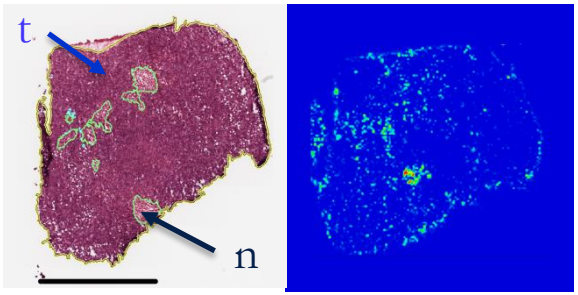


The observed heterogeneity was larger in highest dose level

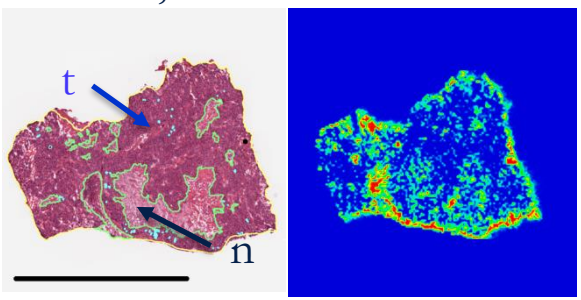


Veliparib low dose (20mg/kg)

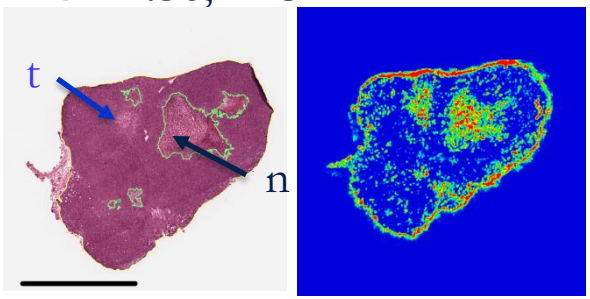
MDA231, ID1



HCC70, ID2

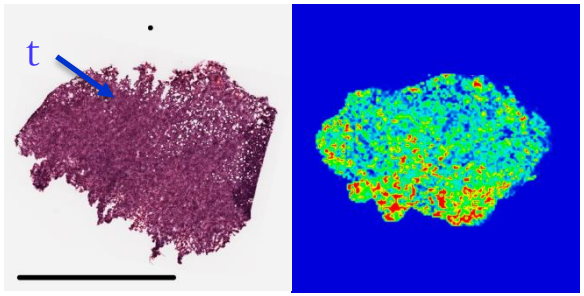


MDA436, ID3

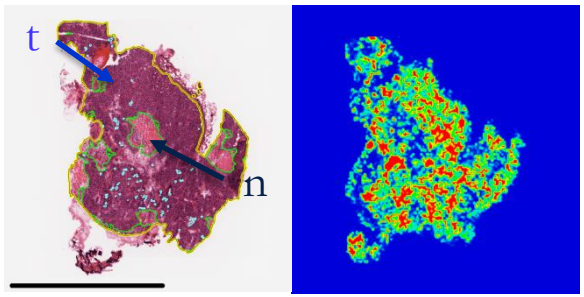


Veliparib high dose (60mg/kg)

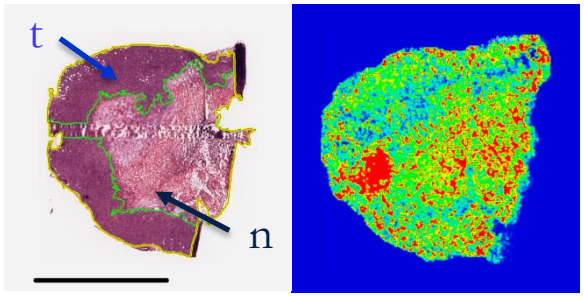
MDA231, ID4



HCC70,ID5



MDA436,ID6

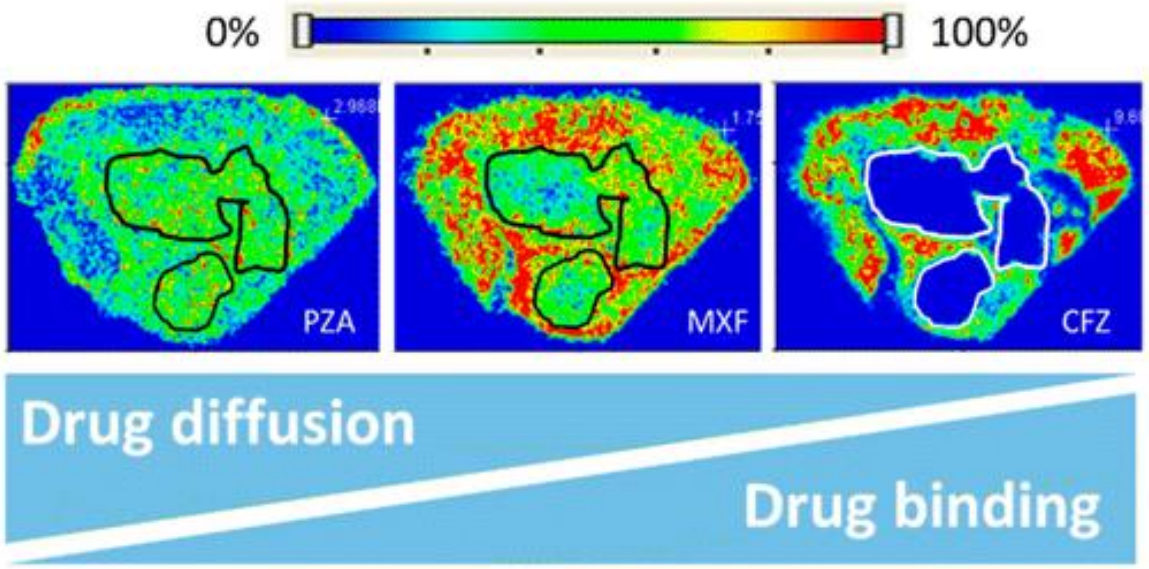


Bartelink IH *et al.* CCR 2016 (submitted)

Drug disposition is dependent on a drug's properties

Lessons from tuberculosis studies

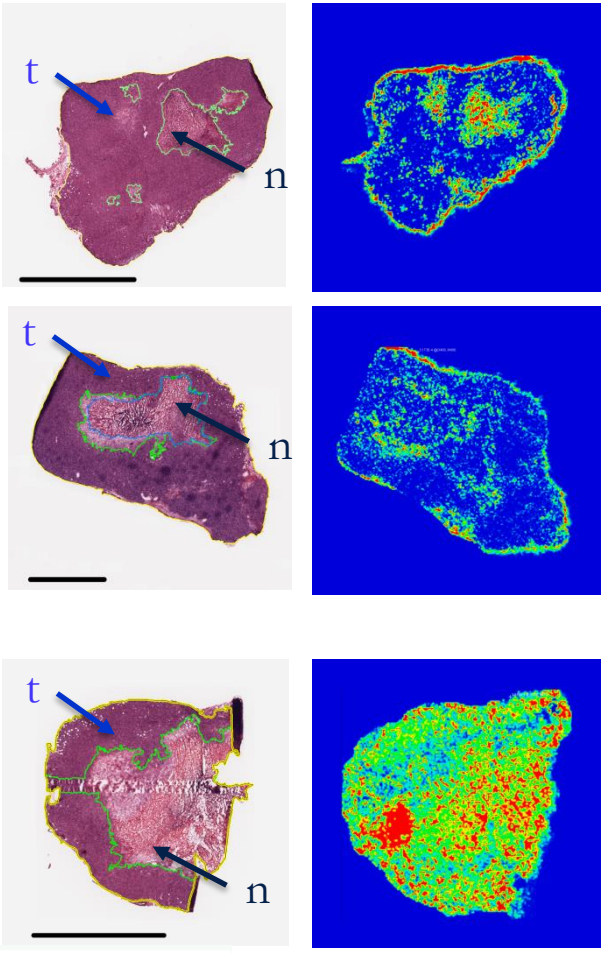
- Screen of 279 drugs: lipophilicity and poor solubility and number aromatic ring predict whether a drug can enter a necrotic region
- Highly relevant for cancers with low vascular, necrotic regions

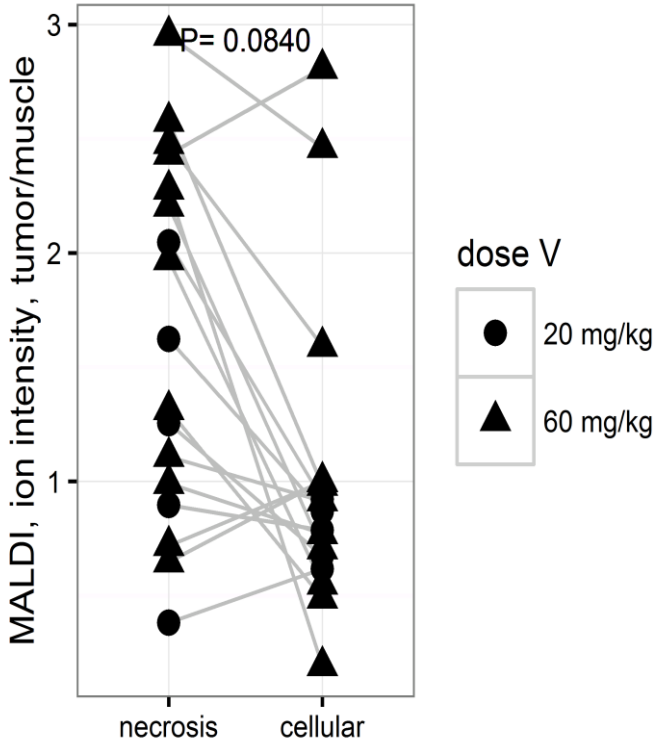


TNBC xenograft study veliparib/carboplatin

Veliparib penetrates into necrotic tissues

Images 2h after administration 20 mg/kg or 60mg/kg dose (at steady state)

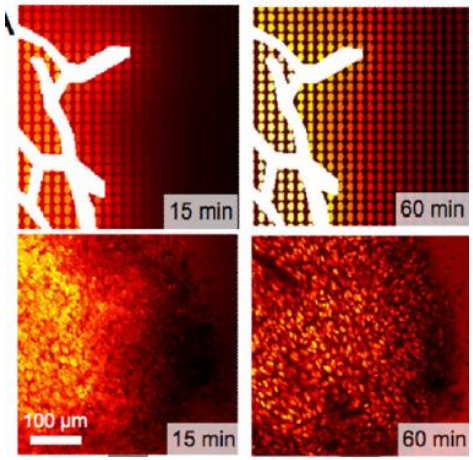


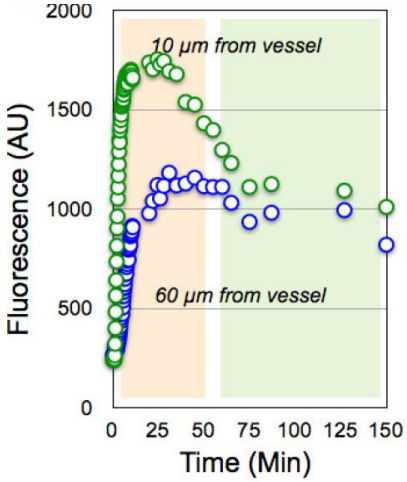


Bartelink IH *et al.* CCR 2016 (submitted)

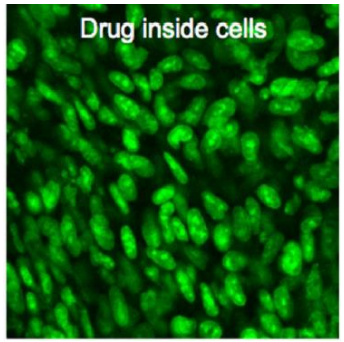
Xenograft study of Fluorescent olaparib
Tumor vessel density may predict drug-uptake

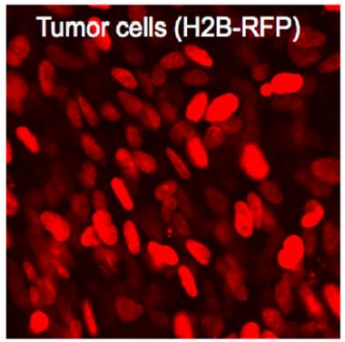
Poorly vascularized regions: large gradient at early time point.

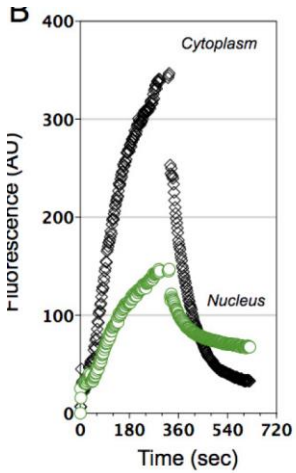




Olaparib accumulates inside nucleus of tum cells.







In vitro uptake

Thurber *et al.* Nat Commun. 4 (1504) 1-20, 2013

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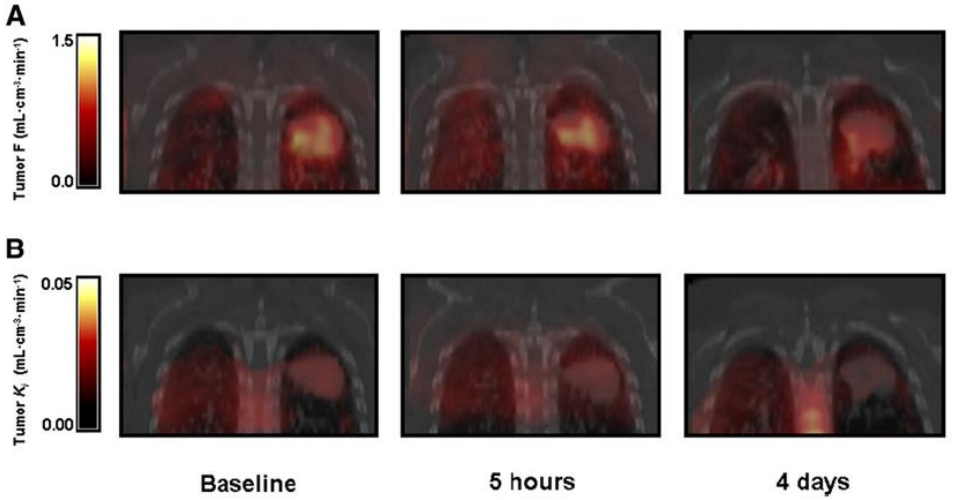
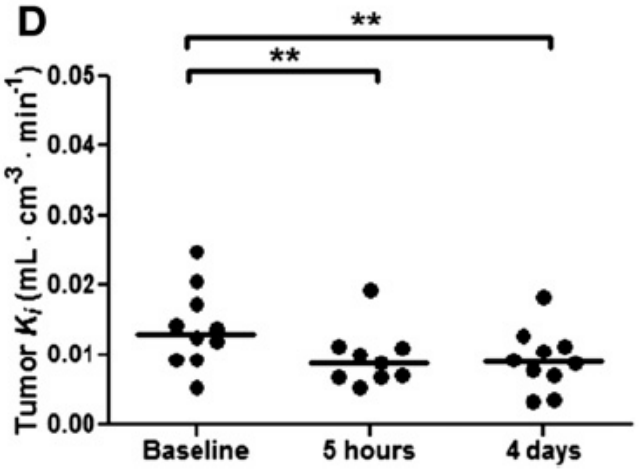
Using imaging to show heterogeneity spatial and temporal drug distribution

- Imaging methods can be used in spatial and temporal distribution in tumor lesions throughout the body
  - Contrast agent enhanced MRI: monitor tumor perfusion/permeability of tumor vasculature/tissue
  - Radiolabeled drug –PET scan drug specific penetrance and heterogeneity in uptake between lesions
  - Non-invasively monitor changes in drug penetrance over time

^{11}C docetaxel in 10 patients with NSLC

Study bevacizumab reduces penetration docetaxel





Day 1 Day 4

Administration:

^{11}C Docetaxel

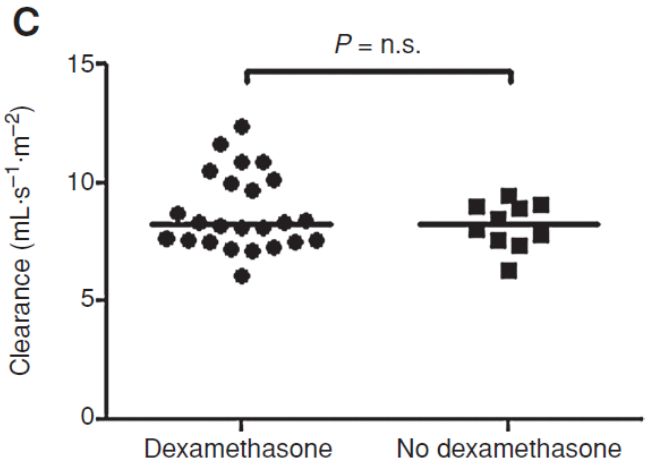
Bevacizumab (VEGF inhibitor)

Veldt *et al.* Cancer Cell 21: 82-91,2012

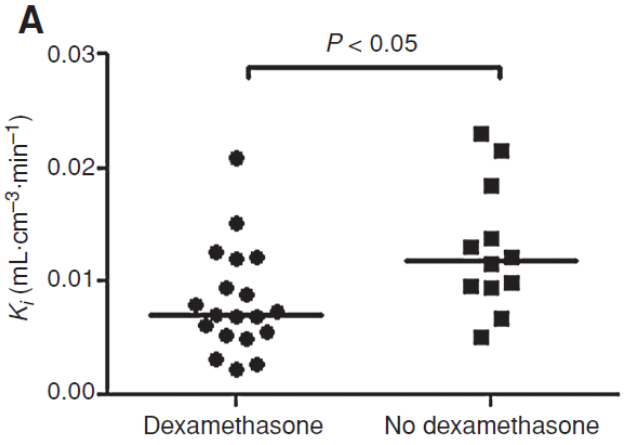
^{11}C docetaxel predicts drug interaction and response in 34 advanced-stage lung cancer

^{11}C docetaxel Uptake in tumor > median significantly better response (RECIST) than < Ki value, P= 0.007).

At similar plasma exposures



Interaction in tumor uptake via OCT3 inhibition?



Veldt *et al.* CCR 17 (14): 4814-4824,2011

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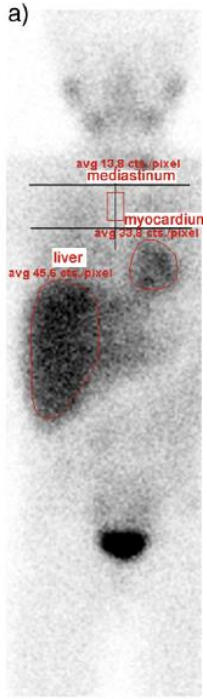
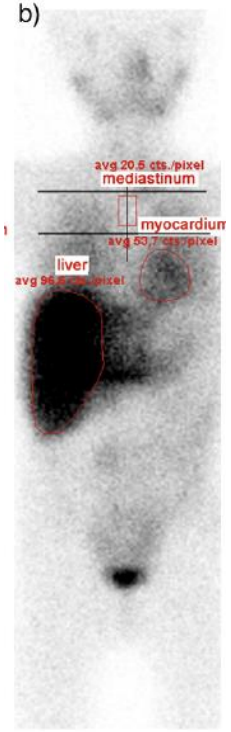
Reversed interaction could improve tracer uptake of ^{123}I mIBG* in neuroblastoma

Scan 3h after **high** dose hydrocortison



Scan 3h after **low** dose hydrocortison



Hydrocortisone in OCT3 expressing cells, the ^{123}I mIBG incorporation is reduced with minor effects on neuroblastoma cells

*mIBG= guanethidine analog that concentrates in sympathetic nervous tissue

Bayer *et al.* NMB43(9):543-551, 2016

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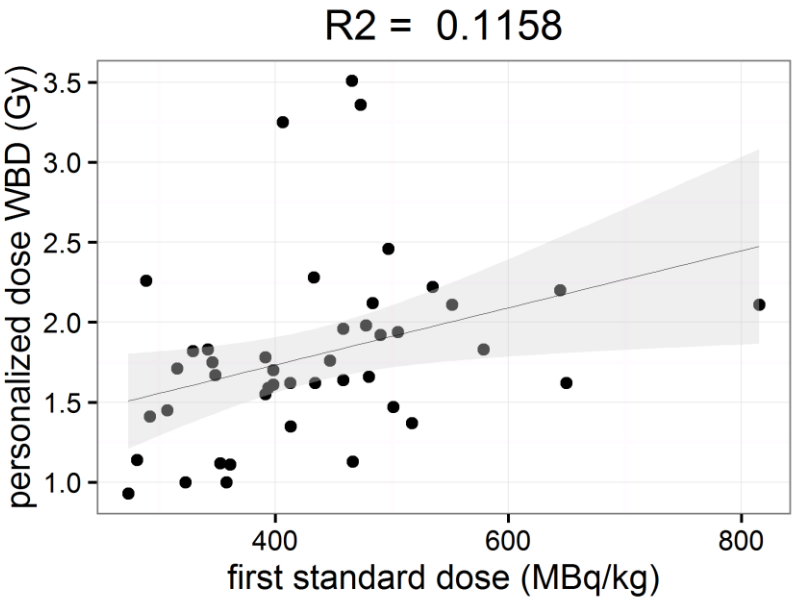
A personalized approach of dosimetry improves outcomes of mIBG treatment

- Whole body dosing (WBD) correlated with lower toxicity
- WBD consistent between consecutive therapies
- WBD does not correlated with MIGB/kg dose



[1]George *et al.* NMC 37, 5, 466-472, 2016

[2] Buckley *et al.* JNM 50,9,1518-1524, 2009

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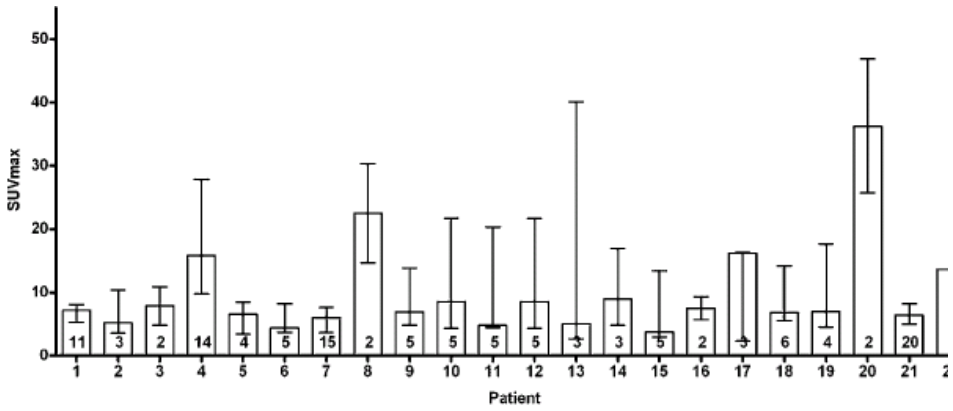
Further potential to personalize of mIBG treatment

- Tumor self-absorbed radiation dose (TSARD) may correlate with higher efficacy
- WBD /TSARD do no correlated with MIGB/kg dose
  - TSARD *versus* MIGB/kg: R^2= 0.32
  - WBD *versus* TSARD: R^2= 0.71, p= 0.0001

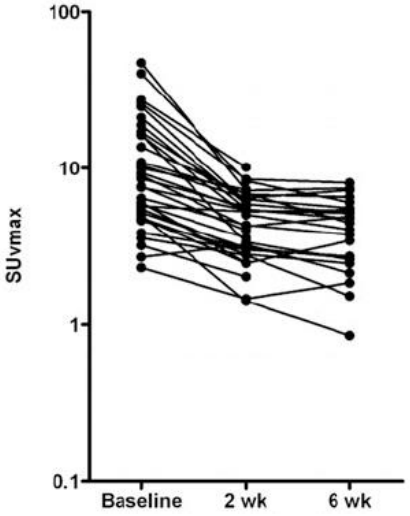
Matthaw *et al.* JNM; 42,1713-172, 2001

89Zr-Bevacizumab PET Visualizes Heterogeneous penetrance in lesions in 22 RCC patients

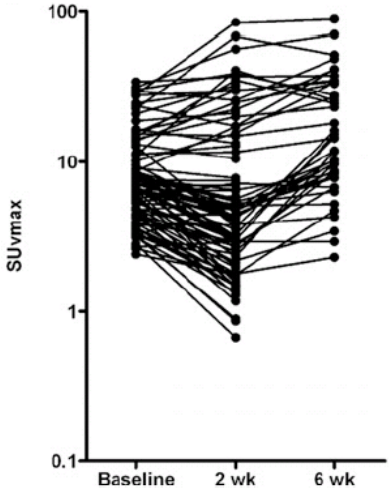
Variability in tumor penetration in lesions at baseline



N=11, bevacizumab/IFNα



N=11,sunitinib



- High baseline tumor SUV_{max} → longer time to progression (HR=0.22 CI95% 0.05-1).
- SUV_{max} did not associate with plasma VEGFa

Oosting *et al.* J Nucl Med. 56:63-69, 2015

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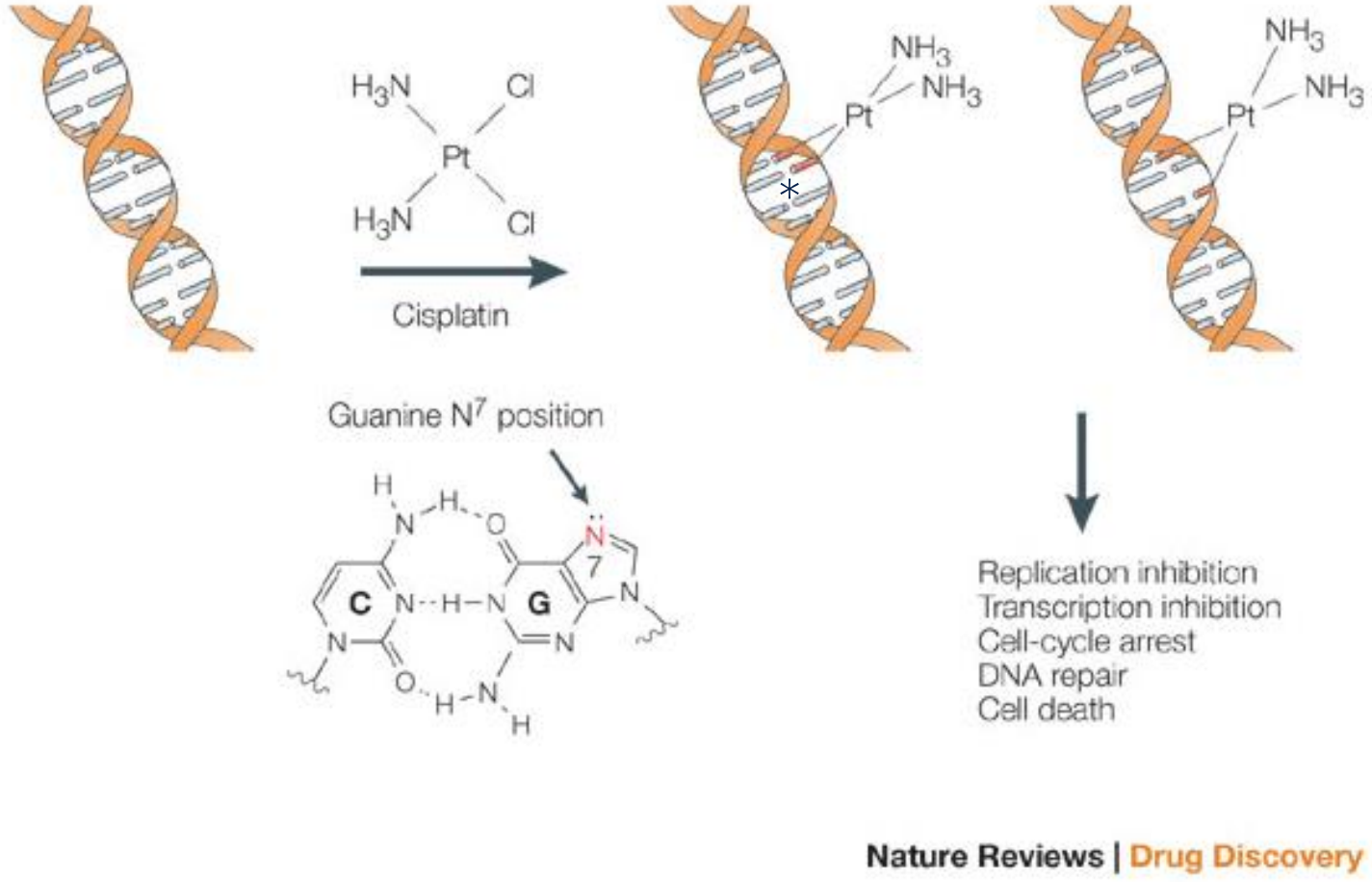
Platinum adduct formation in DNA of target cells may be more informative



Wang and Lippard, Nature Reviews 4, 307- 320, 2005

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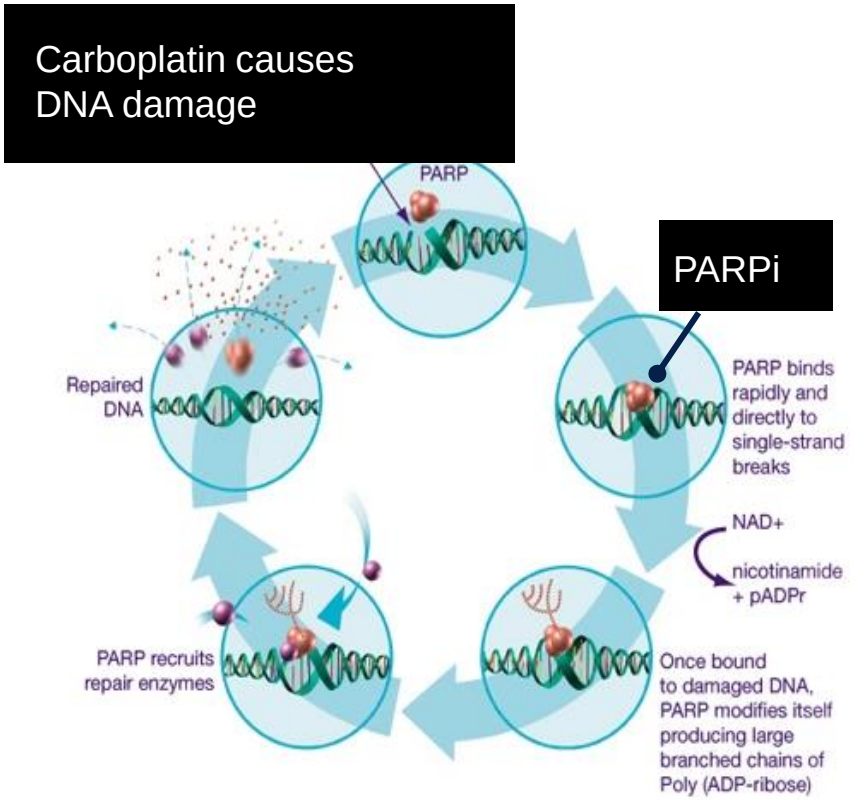
Platinum adduct formation in DNA may accumate at PARPi cotreatment



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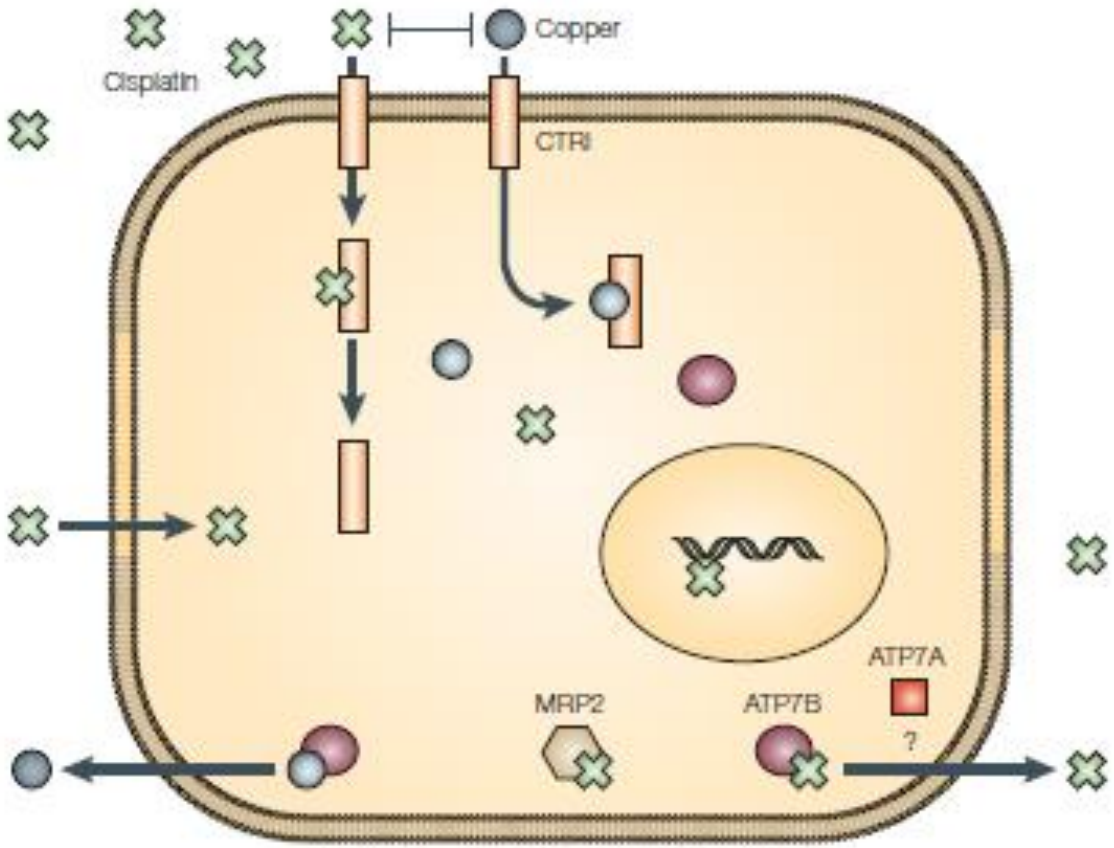
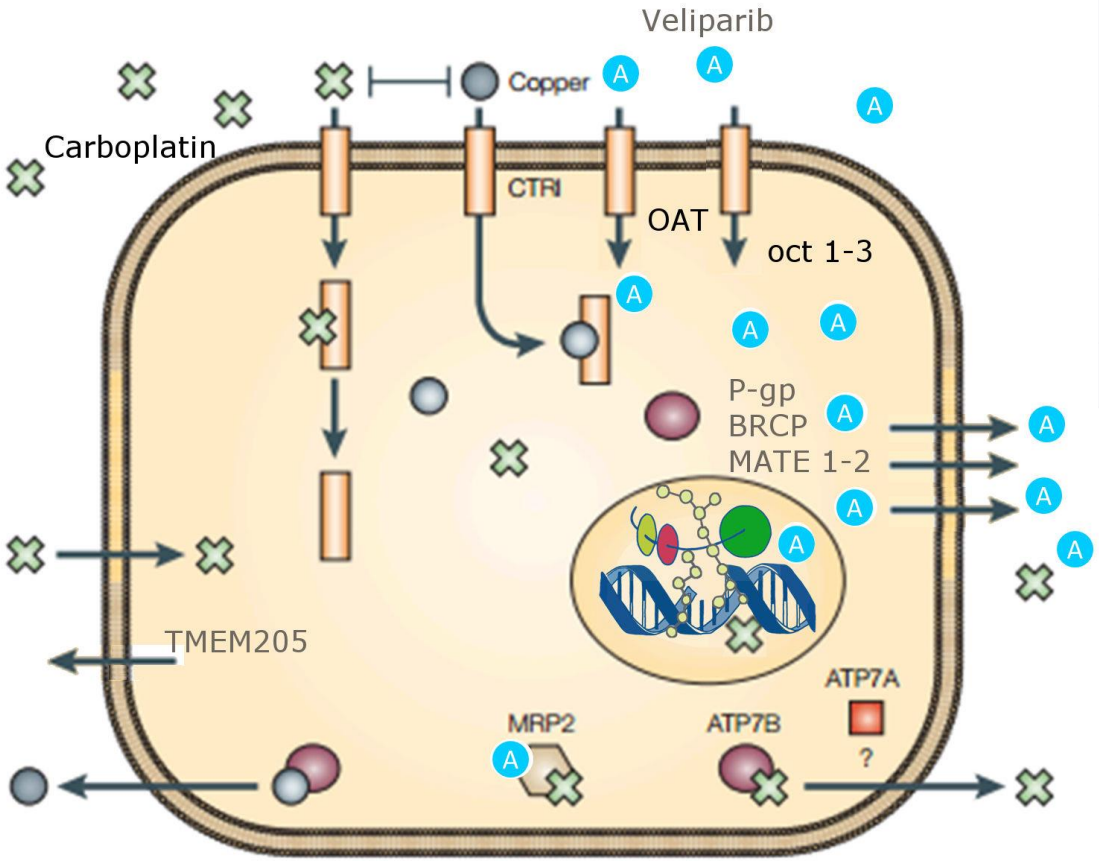
Intracellular adduct formation influenced by drug transporters



Wang and Lippard, Nature Reviews 4, 307- 320, 2005

This is more complex when the platinum is combined with a PARPi



Uptake transporters	Efflux transporters
OCT 1-3	P-gp, BCRP, MRP2/4
OAT 1-3	MATE1-2
CRT1-2	ATP7A-B
	TMEM205

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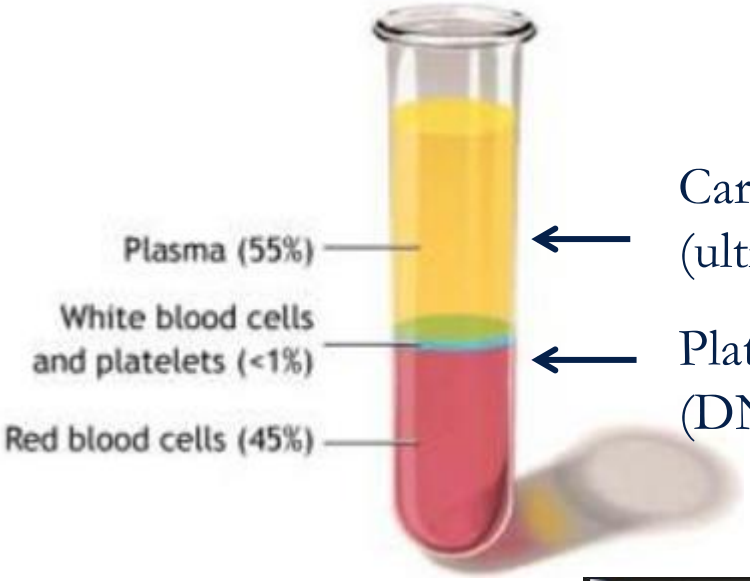
Platinum adducts measurement
in lymphocytes and tumor cells

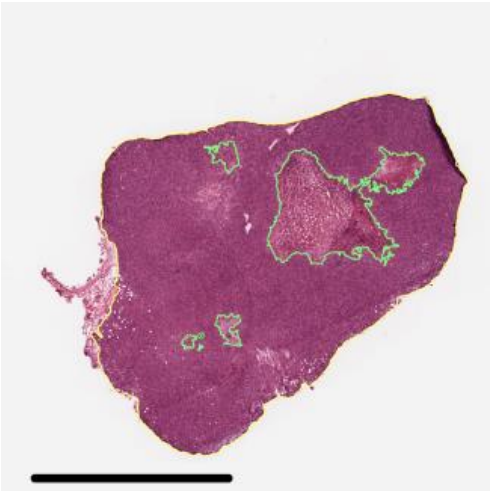
Blood sample

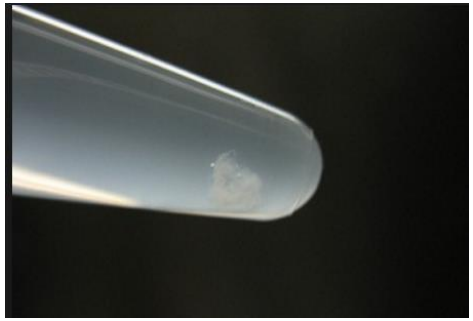


Carboplatin (ultrafiltrate) →

Platinum adducts (DNA extraction) →

Tumor section





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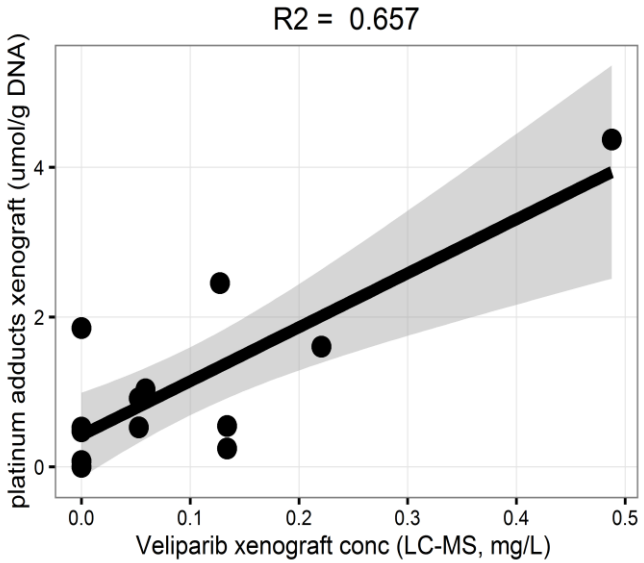
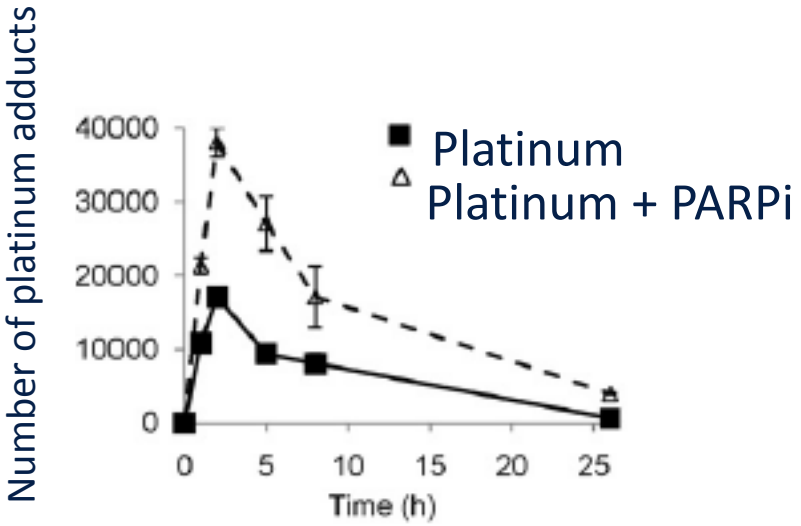
TNBC xenograft study veliparib/carboplatin

Platinum adduct formation may depend on PARPi co-administration





Bartelink IH et al. CCR 2016 (submitted)

Olaussen *et al.* Lung Cancer 80: 216-222,2013

Phase 1 study talazoparib/ carboplatin shows feasibility of measuring intracellular concentrations to predict toxicity

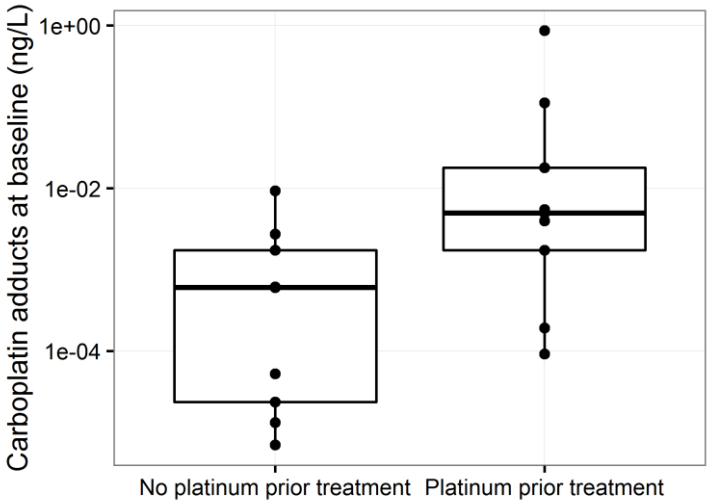
Toxicity	N	%
Neutropenia	**12**	**50%**
Anemia	**10**	**42%**
Thrombocytopenia	**7**	**29%**
Fatigue	**3**	**13%**

Toxicity profile of talazoparib 0.75-1mg /carboplatin AUC 1.5 weekly/2/3 weeks

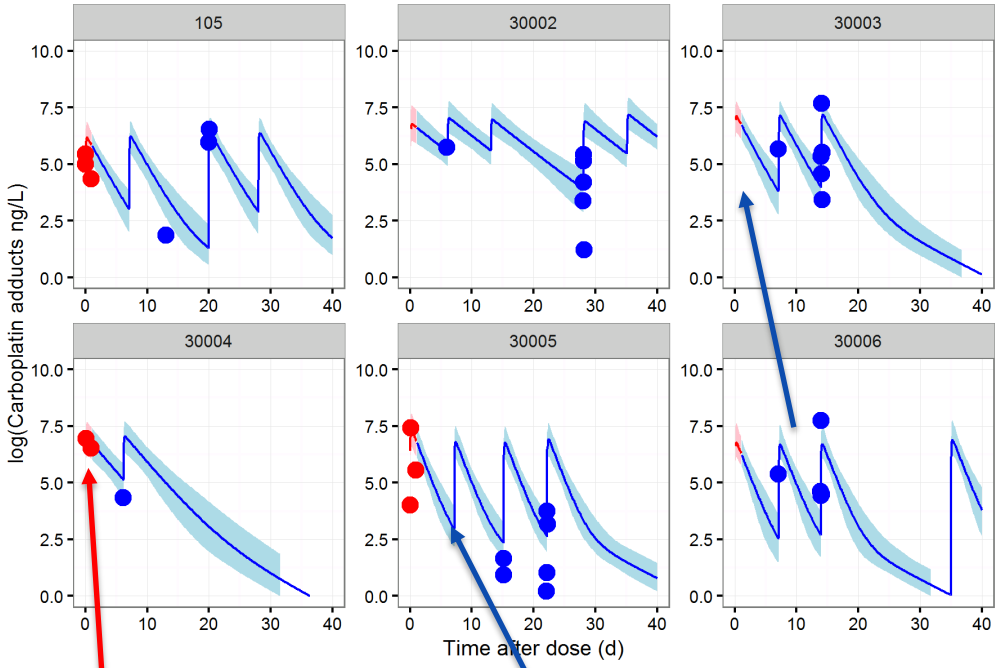
Dhawan M, Bartelink IH. et al. ASCO/AACR abstract 2016

Carboplatin adduct formation in PMBCs not affected by PARPi

Carboplatin adducts long $t_{1/2}$ and large variability



Carboplatin adduct formation did not differ between single and combination treatment



Dhawan M, Bartelink IH. et al. ASCO/AACR abstract 2016

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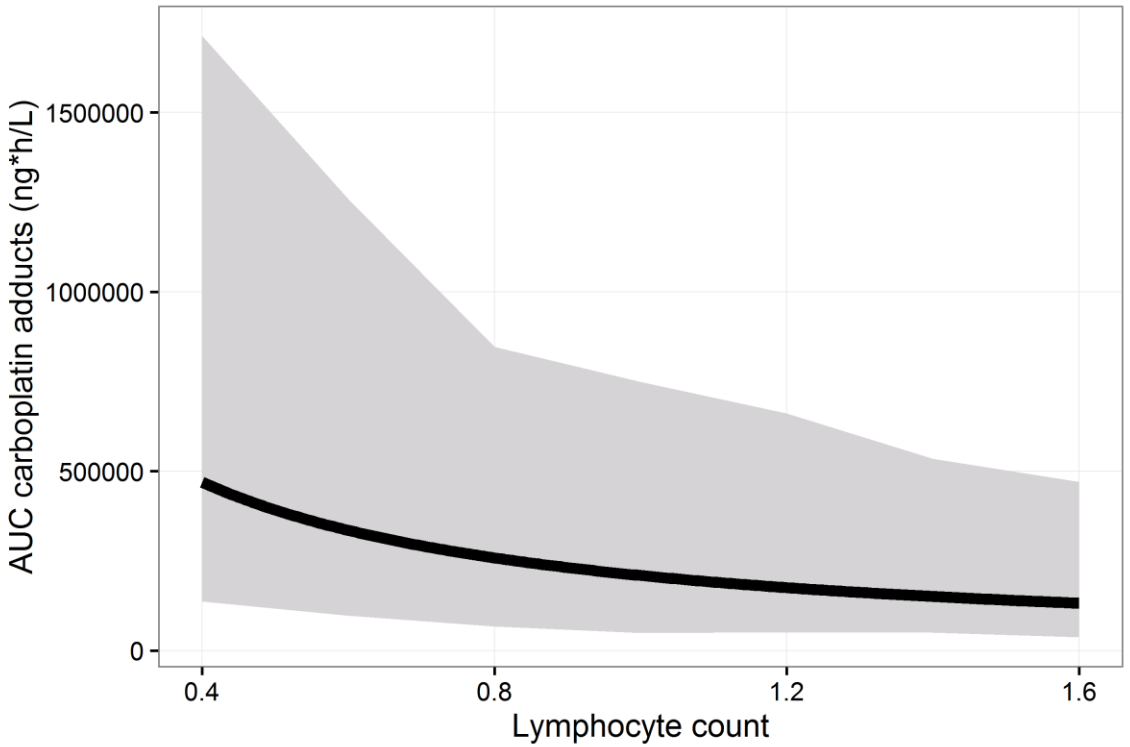
Carboplatin adduct formation may relate to toxicity

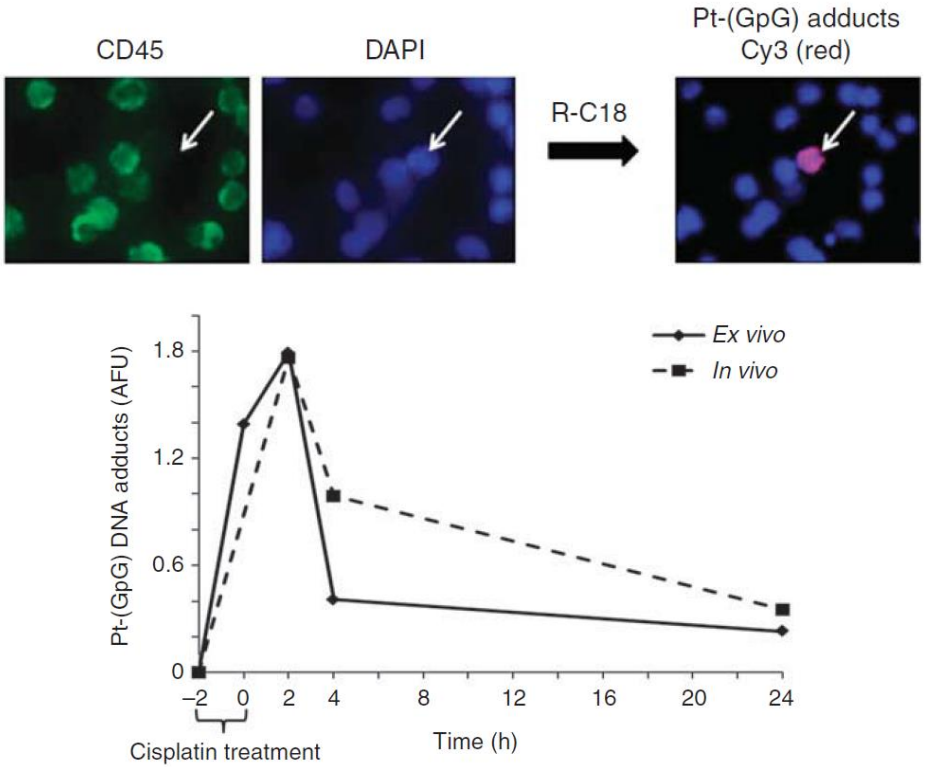


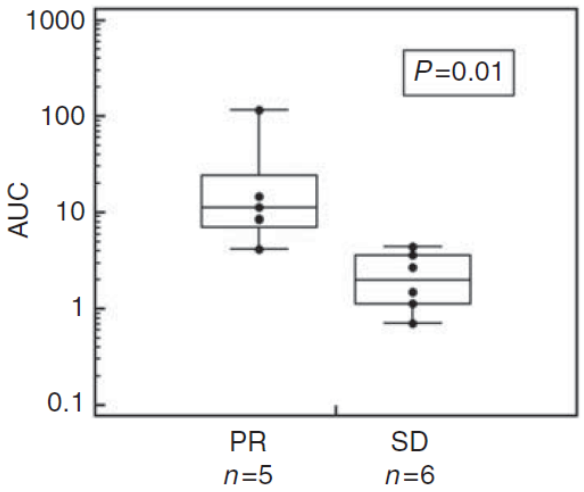
Carboplatin adduct formation higher in patients who are more prone to lymphocyte toxicity

Dhawan M, Bartelink IH. et al. ASCO/AACR abstract 2016

Platinum adduct accumulation ex vivo may predict outcome

Immunofluorescence technique in circulating tumor cells





Ex vivo adduct accumulation in NSCLC patients may predict response (P=0.01; n=11)

Nel *et al.* BJC 109: 1223-1229, 2013

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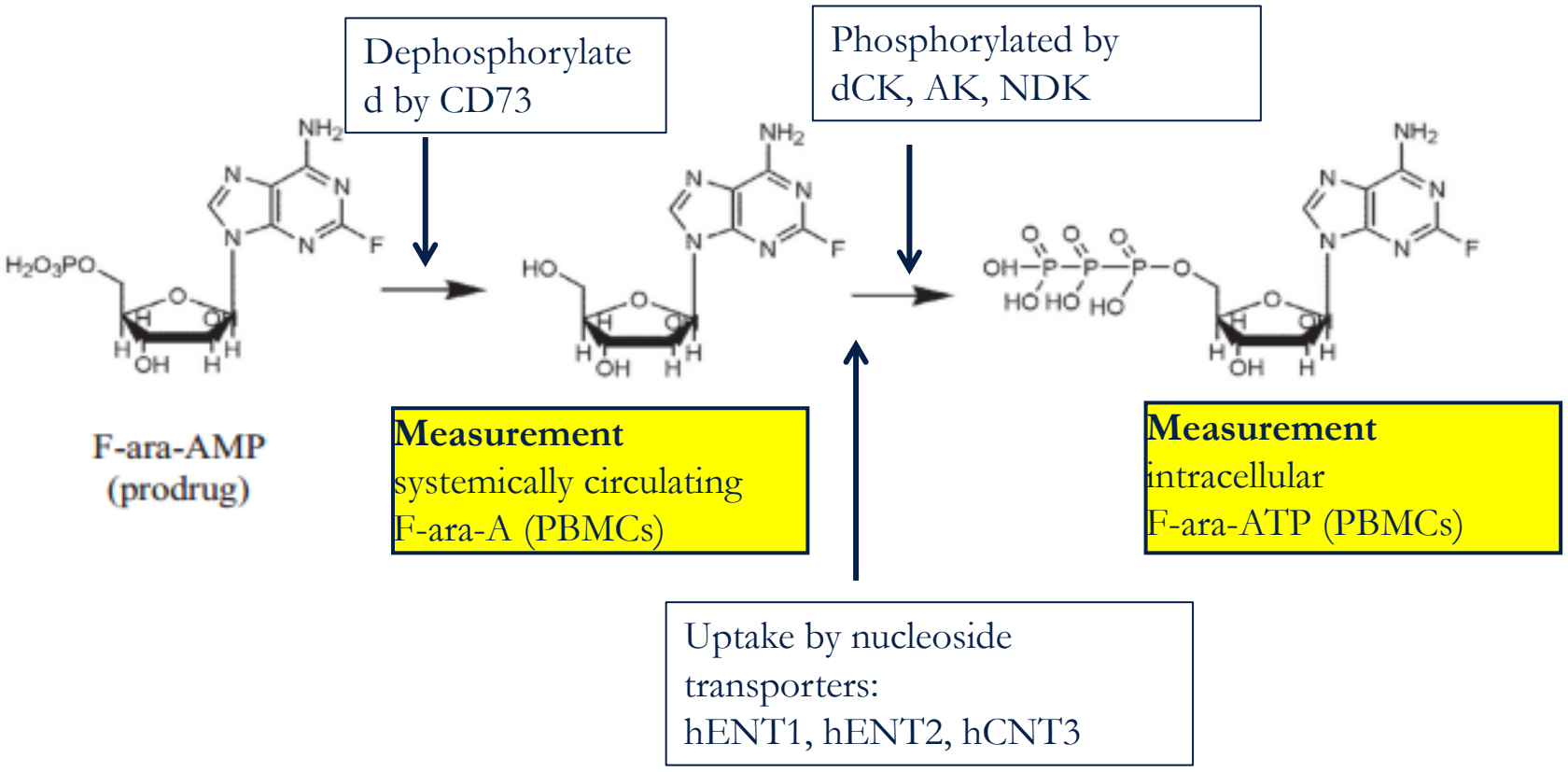
Intracellular measurements of fludarabine in children pre-HCT by LC-MS



Huang *et al.*, JPBA 86 (198): 1-19, 2013
Woodahl *et al.* CCP 63(5):959-64, 2009

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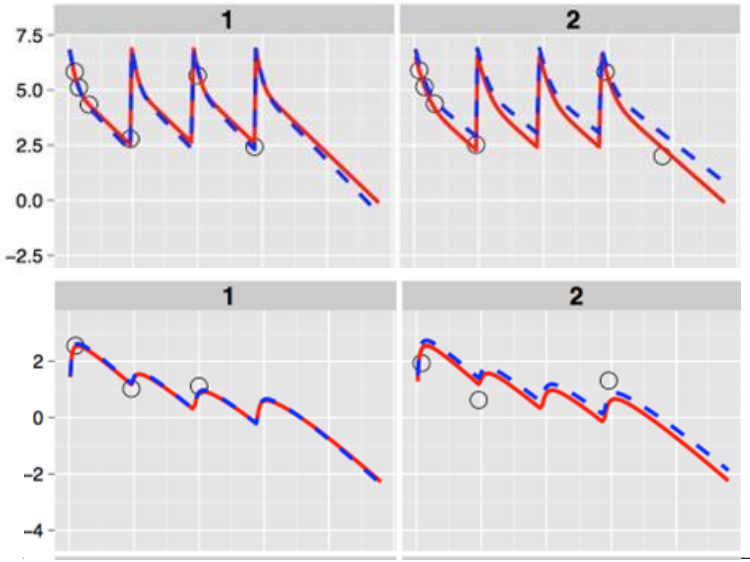
Fludarabine pre-HCT shows temporal differences in intracellular drug accumulation

Study of 133 children conditioned with a fludarabine-based regimen

intracellular f-ara-ATP ↓ with time

f-ara-ATP	Dose 1	Dose 3	Dose 4	Dose 5
2hr post start infusion	9.6 (1-18.2)	3.3(0.41-12)	1.73(0.57-11.6)	0.64(0.22-1.4)
Number of samples	17	16	16	5

Example ID:



Plasma fludarabine no alterations

Intracellular f-ara-ATP ↓ with time

Long-Boyle 2016 in preparation

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Number labeled tracers used in clinical trials for multiple targets

- Ongoing trials in just breast cancer in 2016: **N=164***
- **Any** NIH-registered pediatric trial in 2016 **N=4**

Tracer type	disease	Patient inclusion
^{68}Ga-DOTATOC	brain tumors	suspended participant recruitment
^{18}F-DOPA	CNS Tumors	currently recruiting participants
18F-FLT	Brain Tumors	recruitment on invitation
18F-FLT	Gliomas	withdrawn prior to recruitment

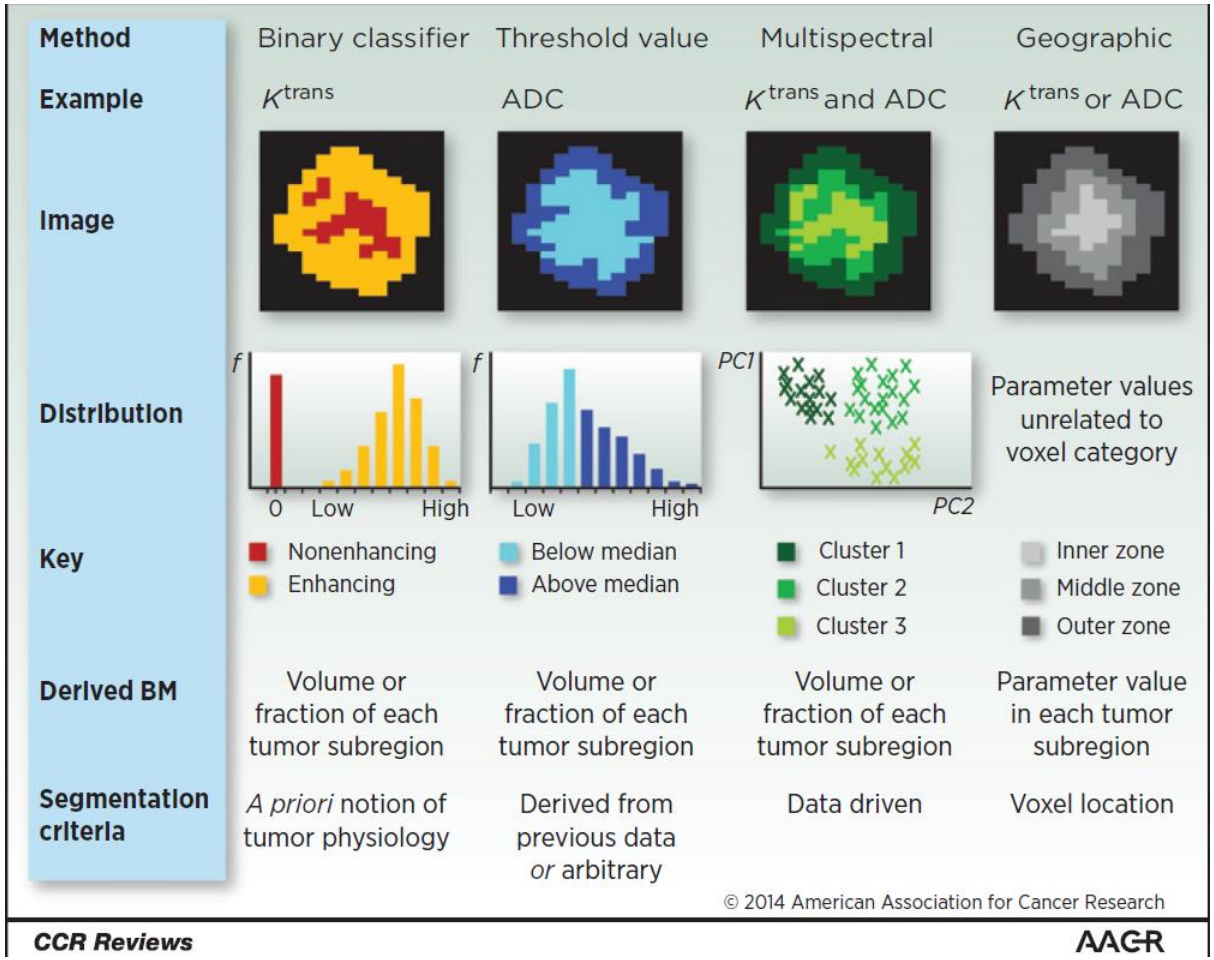
*Van Es, JNM 57(2,Suppl 1):96S-104S, 2016

Barriers for implementation in the clinic in adults and children

- Radiolabeled compounds
  - Investigational medicinal product dossier (IMPD)
  - Randomized clinical trial needed
  - Financial strains
  - Logistics
  - Complex data-analysis
  - Children: fear of radiation (CT-scan + radiolabeled drug)
  - Trial accrual
- Drug measurements in tumor biopsies
  - Biopsies scheduled at the right time after drug administration
  - Advanced equipment needed

Van Es, JNM 57,2,96S, 2016

Advanced imaging techniques may be needed to understand drug penetration



O'connor *et al.* CCR 21(2):250-257, 2015

Conclusions

- Implementation of drug measurements at the pharmacological target site
  - May improve success rate of early phase pediatric clinical trials, especially basket trials
  - Can be used to understand non-response in clinical practice
    - Use micro doses of tracer drugs
    - Take tumor biopsies during drug dosing

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