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
Dosing schedule accounting for age-related pharmacokinetics, example busulfan

1. Dose/m2 or /kg ≠ uniform exposure

Estimate exposure

Pharmacokinetics

Individualized dosing

- Exposure: Area Under the Concentration-time curve (AUC) = dose*bioavailability/Clearance
- Dose optimized= $\frac{AUC_{\text{target}} \cdot \text{Dose}_i}{AUC_i}$

Bartelink IH et al. CPK 51 (5) 331-345, 2012
Other examples of PK-PD based individualized pediatric dosing

- 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, assessment 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
3. Mehrotra et al. DMD #69559, 2016
Demonstrating Efficacy For Oncologic Agents Remains a Challenge

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

Wharton et al. Pediatrics 134 (2) e512-e518, 2015
## Beyond genomics, challenges in pediatric drug development

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

2. Concentration plasma ≠ tumor

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
2. Concentration plasma ≠ tumor

Poor correlation between 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
3. Variability in drug penetrance per tumor type

Drug penetration may differ between tumor types

Large uptake in NSCL

*Study differences:

Sampling time differs slightly
Measurement 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
Teniposide penetration may differ between tumor types

3. Variability in drug penetrance per tumor type

Haura et al. JTO 5:1806-1814, 2010
3. Variability in drug penetrance per tumor type

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)
4. Heterogeneity in spatial + temporal drug distribution

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

Tissue section  Matrix application  MALDI-MSI measurement  Data analysis

Mixed sample matrix  Laser source  Desorption  Ionization  Molecular ions

Internal standard  veliparib

Single ion intensity map

High  Intensity  Low

Ion density maps

MALDI-MSI measurement diagram
4. Heterogeneity in spatial + temporal drug distribution

MALDI-MSI shows heterogeneous veliparib penetration

The observed heterogeneity was larger in highest dose level

MALDI MSI shows heterogeneous veliparib penetration

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
4. Heterogeneity in spatial + temporal drug distribution

**TNBC xenograft study veliparib/carboplatin**

Veliparib penetrates into necrotic tissues

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

![Images showing penetration of veliparib into necrotic tissues](image)

**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 tumor cells.

Thurber et al. Nat Commun. 4 (1504) 1-20, 2013
4. Heterogeneity in spatial + temporal drug distribution

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
11C docetaxel in 10 patients with NSLC

Study bevacizumab reduces penetration docetaxel

4. Heterogeneity in spatial + temporal drug distribution

**11C** Docetaxel

**Bevacizumab (VEGF inhibitor)**
4. Heterogeneity in spatial + temporal drug distribution

$^{11}$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?
4. Heterogeneity in spatial + temporal drug distribution

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*
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

4. Heterogeneity in spatial + temporal drug distribution

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1 George et al. NMC 37, 5, 466-472, 2016
2 Buckley et al. JNM 50,9,1518-1524, 2009
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: $R^2 = 0.71$, $p = 0.0001$
89Zr-Bevacizumab PET Visualizes Heterogeneous penetrance in lesions in 22 RCC patients

Variability in tumor penetration in lesions at baseline

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

N=11, bevacizumab/IFNα  N=11, sunitinib
5. Variability in intracellular uptake and transformation

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

5. Variability in intracellular uptake and transformation

Platinum adduct formation in DNA may accumulate at PARPi cotreatment.
5. Variability in intracellular uptake and transformation

Intracellular adduct formation influenced by drug transporters

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

<table>
<thead>
<tr>
<th>Uptake transporters</th>
<th>Efflux transporters</th>
</tr>
</thead>
<tbody>
<tr>
<td>OCT 1-3</td>
<td>P-gp, BCRP, MRP2/4</td>
</tr>
<tr>
<td>OAT 1-3</td>
<td>MATE1-2</td>
</tr>
<tr>
<td>CRT1-2</td>
<td>ATP7A-B</td>
</tr>
<tr>
<td></td>
<td>TMEM205</td>
</tr>
</tbody>
</table>
5. Variability in intracellular uptake and transformation

Platinum adducts measurement in lymphocytes and tumor cells

Blood sample

- Carboplatin (ultrafiltrate)
- Platinum adducts (DNA extraction)

Tumor section
5. Variability in intracellular uptake and transformation

**TNBC xenograft study veliparib/carboplatin**
Platinum adduct formation may depend on PARPi co-administration

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**Graph 1:**
- **y-axis:** Platinum adducts xenograft (umol/g DNA)
- **x-axis:** Veliparib xenograft conc (LC-MS, mg/L)
- **R2** = 0.657

**Graph 2:**
- **x-axis:** Time (h)
- **y-axis:** Number of platinum adducts
- **Legend:**
  - Platinum
  - Platinum + PARPi
Phase 1 study talazoparib/ carboplatin shows feasibility of measuring intracellular concentrations to predict toxicity

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>N</th>
<th>%</th>
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</thead>
<tbody>
<tr>
<td>Neutropenia</td>
<td>12</td>
<td>50%</td>
</tr>
<tr>
<td>Anemia</td>
<td>10</td>
<td>42%</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>7</td>
<td>29%</td>
</tr>
<tr>
<td>Fatigue</td>
<td>3</td>
<td>13%</td>
</tr>
</tbody>
</table>

Toxicity profile of talazoparib 0.75-1mg /carboplatin AUC 1.5 weekly/2/3 weeks
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
Carboplatin adduct formation may relate to toxicity

Carboplatin adduct formation higher in patients who are more prone to lymphocyte toxicity
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)
5. Variability in intracellular uptake and transformation

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

- Uptake by nucleoside transporters: hENT1, hENT2, hCNT3
- Dephosphorylated by CD73
- Phosphorylated by dCK, AK, NDK

Measurement
- Systemically circulating F-ara-A (PBMCs)
- Intracellular F-ara-ATP (PBMCs)

Huang et al., JPBA 86 (198): 1-19, 2013
Woodahl et al. CCP 63(5):959-64, 2009
5. Variability in intracellular uptake and transformation

**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

<table>
<thead>
<tr>
<th>f-ara-ATP</th>
<th>Dose 1</th>
<th>Dose 3</th>
<th>Dose 4</th>
<th>Dose 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>2hr post start infusion</td>
<td>9.6 (1-18.2)</td>
<td>3.3(0.41-12)</td>
<td>1.73(0.57-11.6)</td>
<td>0.64(0.22-1.4)</td>
</tr>
<tr>
<td>Number of samples</td>
<td>17</td>
<td>16</td>
<td>16</td>
<td>5</td>
</tr>
</tbody>
</table>

Example ID:

Plasma fludarabine no alterations

Intracellular f-ara-ATP ↓ with time

Long-Boyle 2016 in preparation
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 $N=4$

<table>
<thead>
<tr>
<th>Tracer type</th>
<th>disease</th>
<th>Patient inclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{68}$Ga-DOTATOC</td>
<td>brain tumors</td>
<td>suspended participant recruitment</td>
</tr>
<tr>
<td>$^{18}$F-DOPA</td>
<td>CNS Tumors</td>
<td>currently recruiting participants</td>
</tr>
<tr>
<td>18F-FLT</td>
<td>Brain Tumors</td>
<td>recruitment on invitation</td>
</tr>
<tr>
<td>18F-FLT</td>
<td>Gliomas</td>
<td>withdrawn prior to recruitment</td>
</tr>
</tbody>
</table>

*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
6. Implement target site-outcome association

Advanced imaging techniques may be needed to understand drug penetration

<table>
<thead>
<tr>
<th>Method</th>
<th>Example</th>
<th>Distribution</th>
<th>Key</th>
<th>Derived BM</th>
<th>Segmentation criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>A priori notion of tumor physiology</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Volume or fraction of each tumor subregion</td>
<td>Derived from previous data or arbitrary</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>Volume or fraction of each tumor subregion</td>
<td>Data driven</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Volume or fraction of each tumor subregion</td>
<td>Voxel location</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Parameter values unrelated to voxel category</td>
<td></td>
</tr>
</tbody>
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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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