Symposium 10: Translating from the Laboratory to the Patient: Advanced Development Requirements – The Animal Rule and a Clinical Indication

A Walk Through the “Valley of Death” Wearing Scintillating Glasses and TLDs

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What is Advanced Development?

“Advanced Development IS “Attention to Detail”

Quality Assurance ~ Quality Planning ~ SOPs ~ GXP ~ Statistical Certainty (Power) ~ Communication with the FDA
What is Advanced Development?

What does the FLOW of Development Activities for a Drug/Biologic look like:

Idea => RO1 ➔ Early Development ➔ NIAID ➔ Corporate Partnering ➔ BARDA ➔ FDA

The USG uses Technology Readiness Levels (TRLs) to cover the development activities

Research (RO1, others) ➔ Contracted SOWs (timelines/milestones)

The Public Health Emergency Medical Countermeasure Enterprise, or PHEMCE, relies on coordination of multiple contracts are engaged to meet the USG requirements and avoid a “single point of failure” in preparation.

Proof-of-Concept ➔ Advanced Development ➔ Acquisitions

TRL 1-3 ➔ TRL 4-5 ➔ TRL 6-7 ➔ TRL 8 ➔ TRL 9

TECHNOLOGY MATURITY

EUA potential

1 approved
What is Involved in MCM Advanced Development?

What are we going to cover?

A. Important Concepts Explained
   • Dosimetry and the Institutional Lethality Profile
   • What is LETHALITY and when do we bias the outcome
   • The Kaplan-Meier Plot and the PROBIT Plot
   • The Animal Rule
   • Allometrics

B. Early Development - in-vitro signal and the EC50
   • Translation of the EC50 to the Cmax and required exposure
   • The clinical indication

C. Advanced Development – Exploiting the Therapeutic Index (TI)
   • Study “Power” and the magnitude of effect
   • In ARS – understanding the PK, Biodistribution, and the F-value
   • Clinical safety trials

D. Operational use of Countermeasures
   • The SNS and CONOPs, the Government emergency need vs Commercial need
• We all understand this figure …or do we?
• The ionizing radiation we work with is from multiple sources, multiple energies, multiple measures of dosimetry, and the biology is complicated by the physical chemistry, depth dose distribution, and the highly variable sensitivities of the biologic target
Radiation Injury – Measure the Dose

• **IR Methods with Quality Measures**
  
  – What does a Gy unit mean in our work?
  – It is an impact of energy as joules per kg.
    - 1 Gy = 1 J/kg
  – 1 Rad = 100 ergs/gm = 1 cGy
    - 1 Gy = 100 rads

• Tissue absorption is a specific property and thus we have a **spectrum of absorbed doses** across the affected body. We claim a unit dose of Gy as a unit of “an average” absorbed energy

• **but in reality it is very noisy** (can be ± 50%)

• LINAC (6 MV), Co-60, Cs-137 and X-ray

• They are all ionizing

• They are all **DIFFERENT**

It would be “easy” if we were a “balloon of water” but we are a mix of densities. We make “assumptions” and live with that “noise”
• Inhomogeneity of the depth dose
  • the body is a mix of densities and TBI is not performed on a uniform slab of “meat”
  • Hounsfield units (HU) measure density
• Bilateral TBI is not like “flipping burgers on the BBQ” - dependent on the incident energy
• Thermal penetration is not ionizing penetration
• The depth dose distribution of X-rays, Cs-137, Co-60, LINAC (2MV & 6 MV) are different systems

These are **homogeneous** targets:
- Rare
- Medium Rare
- Medium
- Medium Well

These are **inhomogeneous**:

Appreciate the biologic “noise”
IR Methods and Quality Assurance

- One needs to understand the radiation source dosimetry in terms of field uniformity, quality, build up region, depth dose distribution, dose rate, and Kerma

“Kerma”: “dose of ionizing radiation in a sample of matter divided by the mass of the sample” …..that is… density matters

….and Energy matters

- Consider the build-up region

Tools for Good Dosimetry:
- Farmer probe (NIST certified)
- TLDs, MOSFETs, Diodes, etc.
- Superflab (attenuation media)

Entry and Exit Measures:
- Know the depth dose distribution
- Appreciate the biologic “noise”
• Lethality is the endpoint of choice for the FDA in Animal Models
  • Why? For the approval pathway it is the clearest endpoint
  • Reduced/mitigated lethality allows for additional medical management

• What is lethality? Many causes:
  • Septic death, coagulopathies, oxidative DNA damage, metabolic failures, ....all of the above and more - Control what you don’t want to pollute your data
  • Lethality can also be from a bias in the euthanasia criteria or poor training on the criteria implementation
  • We are guided by IACUC and ethical considerations – a necessary bias
  • We make choices on survival based upon euthanasia criteria
    • Important to have consistent criteria and clear clinical rulings

• Solutions:
  • Training…..training…..training…documentation…documentation
  • Blinding the decision makers is a possible control of a selection bias
  • Properly powering a study reduces random selection error
The Institutional Lethality Profile (ILP) is a “bioassay”

- Like any bioassay it has detection limits and confidence boundaries
- It is your “standard curve” for assigning a lethal dose
- The lethality profile needs to be highly reproducible
- It should be reproducible over time and staffing changes
- Animal supply changes, quarantine duration, new instrumentation (irradiators or detectors) must be re-qualified as an integral package and not just individually; the system is an “Institutional” outcome

Construct of the Profile:

- Typically one can use 5 groups of 8 per group (1 animal = 12.5% change)
- 12 animals per group equates to an 8.3% change
- 20 animals/grp is a 5% change - higher “N” = better control

- **Cannot use 100% survival OR 100% lethality – both are null points**
  - Thus one wants five doses each with a surviving fraction
Measuring Lethality – The PROBIT Plot

Defines the Working Range for your survival outcomes


<table>
<thead>
<tr>
<th>Dose, rads</th>
<th>Percent</th>
<th>Probit</th>
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<tbody>
<tr>
<td>565</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>580</td>
<td>4</td>
<td>3.249</td>
</tr>
<tr>
<td>615</td>
<td>7</td>
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<td>650</td>
<td>23</td>
<td>4.261</td>
</tr>
<tr>
<td>665</td>
<td>28</td>
<td>4.471</td>
</tr>
<tr>
<td>680</td>
<td>49</td>
<td>4.975</td>
</tr>
<tr>
<td>700</td>
<td>61</td>
<td>5.279</td>
</tr>
<tr>
<td>715</td>
<td>76</td>
<td>5.706</td>
</tr>
<tr>
<td>745</td>
<td>85</td>
<td>6.036</td>
</tr>
<tr>
<td>800</td>
<td>92</td>
<td>6.405</td>
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</tbody>
</table>

Key Probit Values:
- LD70 = 5.62
- LD50 = 5.00
- LD30 = 4.48

*Important Point:*
The LD100 and LD0 are *not* plot-relevant doses

PROBIT provides a Dose Range Confidence Limit: for the ~LD20 to ~LD80)
Kaplan-Meier “Fantasies”

- The plot below is a good example of the K-M plot for this “Study End”
- The step function indicates a high “n” in the plot groups
- The end time has an appropriate “Δ” where, within the linear range response of the K-M, we see an approximate 30% increase in survival (LD70/x → LD40/x)
Kaplan-Meier “Fantasies”

- Now change the Study End Time. The plot below is now a marginal example.
- The Control group is outside of the “linear range” (> confidence interval for the dose).
- The end time exhibits a survival of approximately 30% (LD70/x → LD40/x).
- But we **HAVE LESS CONFIDENCE** the Control is accurate.

![Kaplan-Meier survival curve diagram](image)
Kaplan-Meier “Fantasies”

• The plot below is an **UNACCEPTABLE** K-M plot.
• The Study End has survivals that do not reside in the linear range.
• The selected control dose for the ‘assay’ as well as the Treatment response, are outside the linear PROBIT lethality boundaries of LD30 ➔ LD70.
Kaplan-Meier “Fantasies”

- The K-M plot on the left is to be compared to the examples on the right:

  **Top right**: Low “N”; reasonable plot except the drug may be eliciting toxicity or the power is too low (coin toss)

  **Bottom right**: a murine 30 day survival at two MCM doses fails to have sufficient survival; supra-lethal IR dose
• What does it mean to “Power” a study? “Power” reduces random decisional errors that can bias the study in favor of a hypothesis

• $1-\beta$ is the statistical power using alpha as the fractional remnant off the control that you wish to assure the shift exceeds, i.e. $\alpha/2 = 0.025$ (2.5%, 2-sided test)

• Inherent in a low power study is the possibility of a “coin toss” (50/50) outcome where $1-\beta$ is too small.

• Powering a study assures limiting the inherent randomness and is a push on the population effect over individual effect

No MCM vs MCM Rx
Are they “different”? And, at what critical level?

Properly powering a study:
• Reduces the random selection error
• Defines if a “truth” is likely “real”
• To accomplish this we must have a sufficient “n” to rule out “random”
A study shall be adequately powered to assure the study outcome is “real”
If we believe the MCM provides a survival advantage of 25% - what do we need?
Assume an LD50/x Control is taken to an LD25/x by the drug administration:
To attain a survival increase of 25% (LD50/x → LD25/x), a POWER of 0.7, an alpha 0.05
Requires 34 animals per group
Drug Development 101

The in-vitro model – the importance of an established EC50

- provides an effective exposure that elicits a predictive effective (EC) change
  - Assigns a specific plasma concentration to elicit a response
  - Assigns an exposure period (square-wave ON/OFF function)
  - Targeting of the drug depends on the molecular mechanism; cell-surface receptor (easier) vs nuclear envelope or nuclear protein (harder)
  - Receptor affinity should be defined with an Emax, a rate to Emax, vs dose

![Graph showing Emax and EC50 relationship](image-url)
The in-vitro model – the importance of an established EC50

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  - Assigns a **specific plasma concentration** to elicit a response
  - Assigns an **exposure period** (square-wave ON/OFF function)
  - Targeting of the drug depends on the molecular mechanism; cell-surface receptor (easier) vs nuclear envelope or nuclear protein (harder)
  - Receptor affinity should be defined with an Emax, a rate to Emax, vs dose

**Maximal response**

**Translate to the PK Cmax**

\[ E = \frac{E_{\text{max}} \times C}{E_{\text{max}} + C} \]
EC50 to In-Vivo Pharmacokinetics

The EC50 establishes how we need to dose – the effective AUC
EC50 to In-Vivo Pharmacokinetics

The EC50 establishes how we need to dose – the effective AUC

- Maximum Tolerated Concentration (MTC)
- Minimal Effective Concentration (MEC)
- Therapeutic Range
- Intensity of action
- Duration of action
- Absorption rate = elimination rate
The drug dose exposure that creates an efficacious response (increases survival) in the animal model should be correlated to an EC50 “exposure”.

- Drug Clearance (elimination) generally follows body weight or surface area:
  - Clearance is highest in the Mouse > Minipig > Dog > NHP > human

**What is exposure?** Dose x time  Absorption ➔ Elimination varies across species

In general – Human dose = 1x  NHP = 3.1x  Minipig = 1.1x  Dog = 1.8x  Rabbit = 3.1x  Mouse = 12.3x

### Table 1: Conversion of Animal Doses to Human Equivalent Doses Based on Body Surface Area

<table>
<thead>
<tr>
<th>Species</th>
<th>To Convert Animal Dose in mg/kg to Dose in mg/m², Multiply by kₘ</th>
<th>To Convert Animal Dose in mg/kg to HED in mg/kg, Either:</th>
<th>Divide Animal Dose By</th>
<th>Multiply Animal Dose By</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>37</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Child (20 kg)</td>
<td>25</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Mouse</td>
<td>3</td>
<td>12.3</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Hamster</td>
<td>5</td>
<td>7.4</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>6</td>
<td>6.2</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Ferret</td>
<td>7</td>
<td>5.3</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>Guinea pig</td>
<td>8</td>
<td>4.6</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>12</td>
<td>3.1</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>20</td>
<td>1.8</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>Primates:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monkeys</td>
<td>12</td>
<td>3.1</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>Marmoset</td>
<td>6</td>
<td>6.2</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Squirrel monkey</td>
<td>7</td>
<td>5.3</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>Baboon</td>
<td>20</td>
<td>1.8</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>Micro-pig</td>
<td>27</td>
<td>1.4</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td>Mini-pig</td>
<td>35</td>
<td>1.1</td>
<td>0.95</td>
<td></td>
</tr>
</tbody>
</table>

Ref: Guidance: July 2005
Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers

[www.fda.gov/drugs/guidancecomplianceregulatoryinformation/guidances/ucm065014.htm](www.fda.gov/drugs/guidancecomplianceregulatoryinformation/guidances/ucm065014.htm)
Drug Development – The Animal Rule

The Food and Drug Administration issued a final rule in May 2002 to permit the Agency to approve drugs or license biological products on the basis of animal efficacy studies for use in ameliorating or preventing serious or life-threatening conditions caused by exposure to lethal or permanently disabling toxic biological, chemical, radiological, or nuclear substances.

This “Rule” has an update issued May 2014:

Emphasis: Exposure to the human population (AUCpop) must exceed the animal species efficacious dose.
The Animal Rule – Two Requirements

- A Representative Dose-Response Curve for Survival Based on Four Doses of an Investigational Drug Studied in a Well-Characterized Animal Model
  From: Fig 1, Pg 27 Animal Rule (rev; May 2014)
- The Institutional Lethality Profile (ILP) describes the confidence boundaries for a predictive absorbed dose.
- BARDA requires use of the linear portion of the PROBIT curve for efficacy.

Comparisons of Animal and Human PK Data to Support the Selection of an Effective Dose in Humans From: Fig 2, pg 28 Animal Rule (rev)
- The human PK profiles must exceed the efficacious dose in the animal models per figure 3 (Dose #3) where the AUC (population) is above the individual animal measured exposures
• The extent of drug binding to proteins in the plasma can influence distribution characteristics. Only free, unbound, drug can be distributed from the vascular space into other body fluids. A snapshot in time is represented as:

![Concept of Drug Distribution within the Body:](image)

• Each of these can be considered a “compartment” and each has an input and an output represented as “rate constants”. PK models exploit the changes in these rate constants as:
• The exposure of an MCM for Efficacy also has an exposure-related Toxicity (defines the Therapeutic Index, or TI)
• The drug clearance is important and is related to the functionality of the clearance mechanisms following irradiation
• Drug distribution is influenced by a variety of drug-specific physiochemical factors, including the role of drug transporters and protein binding, pH, and perfusion in blood and tissue
• The extent of drug binding to proteins in the plasma (i.e. albumin) can influence distribution ---- Only free, unbound, drug can be distributed from the vascular space into other body fluids.
PK and PD of 2\textsuperscript{nd} Messengers

- The exposure of the drug may trigger \textit{2nd messengers} (SMs) as the MOA
- The AUC of the 2\textsuperscript{nd} messenger may be the AUC of interest in efficacy
  - Follow the chain of activities to describe the MOA
- The expression kinetics and biodistribution of 2\textsuperscript{nd} messengers matters:
  - Example: Cytokine administration (IL-2; Proleukin\textsuperscript{TM}) affects hematopoietic DNA synthesis with daughter cell distributions and tissue infiltrations
  - Images: C-14 thymidine uptake after IL-2 administration
    - Naïve (No IL-2) (B) at 48 hrs versus massive expression of hematopoietic DNA synthesis with infiltrate of daughter cells in the liver, lung and spleen at 48 hr (C) and 72 hrs (D) post IL-2

<table>
<thead>
<tr>
<th>A. anatomy X-sec</th>
<th>B. naive @ 48 hrs</th>
<th>C. 48 hrs post</th>
<th>D. 72 hrs post</th>
</tr>
</thead>
</table>

- Normal thymidine uptake in bone marrow and GI tract
- Heart and blood voids (no cells in circulation and no heart infiltrate)
• H-ARS is a classic, well studied pathology
• Animal models are helping us understand the Natural History (Nat Hx) of biomarker expression changes
• Classic marker: The **Andrews Scale** for lymphocytes is a well known biomarker and is a reliable “biodosimeter” for whole body exposure.
• Selection of the use of No, Minimal, or Full Supportive Care depends on the MCM mechanism of action
• BARDA has used Minimal Supportive Care (MSC) (antibiotics and fluids) as a population baseline of care (with flexibility to add care) to help define the operational limits for MCMs, knowing that some resources and supportive care elements may be scarce immediately after an event.
• Radiation accident use ≠ mass casualty use
• Field use likely requires a Therapeutic Index (TI) > 5
  • Scarce medical management will likely be in effect for several days post a nuclear event making management of adverse events very difficult
Lethality at the hematopoietic doses of radiation is a result of sepsis, coagulopathies, wasting, and other causes that can be controlled by supportive care.

Plots of circulating cell depletion and recovery need to be on a log y-axis versus time in days.

Plots of the survivors alone needs to be provided – avoids the noise from the clinically “at risk” animals.

Blood sampling frequency need to be carefully considered as well as total blood volumes over time as both can compromise lethality.
Gastrointestinal Injury

- GI-ARS is a malady which has been studied as a separate, higher dose-driven pathology.

- Crypt cell death and villus blunting lead to malabsorption syndromes, wasting, bacterial translocation and demonstrates atrophic histology.

- Survival of this injury is poor using the standard hematopoietic time frame (NHPs die in <10 days vs hematopoietic at D12-20 days).

- Many studies define MCM success as a delta in days of survival – BARDA, with FDA concurrence, needs to see survival out in time with demonstrated recovery of GI function.

- The GI radiation injury model is still a work in progress using the NHP, the MP, the rabbit and murine models.

Solutions to Improved GI Studies:

- Use GI injury with preservation of hematopoiesis and chest organs.

- Map the Nat Hx with biomarkers to attain measurable crypt and villus injury (quantitative histology).

- Evaluate the return of functionality (absorption, motility, etc) using non-invasive markers over the D20-D60 post IR (actual times TBD).

Use the high hematopoietic range.
Measuring Gastrointestinal Injury

- **50% shielding**, limiting hematopoietic depression, with lung/heart protection
- **Natural Hx Goals**: Measurable crypt and villus injury using biomarkers vs time
  - **Mitosis** = in-vivo pulsed administration of BrdU (D5-15); necropsy – Ki67; tissue IHC
    - BrdU kinetics – pulse chase type approaches transit analysis and gap separations
    - Ki67 -- IHC post mortem staining of mitotic activity enzymes
  - **Apoptosis**: IHC staining: TUNEL, Caspase, p53, COX-1 and COX-2, others
  - **Vascular endothelial damage markers** – new approaches - TBD
  - **Functional markers** of recovery; specific segment absorption, transit time, intestinal pressures, and GI immuno-competence (GILT regions) over D1 through to D30-D60.
  - **Endotoxin** appearance (breakthrough) and blood sterility
  - **General Histologic architecture**: Pleica circularis flattening, villus height, crypt-villus distances
  - **GI segment comparative analysis**
Mitotsis = in-vivo pulsed administration of BrdU (uracil analog) for crypt viability

BrdU kinetics may be useful in defining the acute injury phase vs controls
  – Gap analysis: Day -3 vs 2 hours before euthanasia — image @ 40X magnification in the crypt
MCM Drug Development "Details"

- The Development Path for an MCM “Drug” or “Biologic”:
  - Identify the proposed molecular pathway in an ARS syndrome
  - Provide an in-vitro model that describes an exposure-effect relationship
  - Provide a stable GMP product which meets target product profile specifications
  - Assign an indication where the MCM provides mitigation, i.e. Hematopoietic syndrome, arrest neutropenia, post-exposure (-24 hr), under a specified drug exposure

- Often Missed Step-by-Step Advancements – learn and implement
  - Develop assays for your product in the test animal blood (sera/plasma);
  - Determine the pharmacokinetics (PK) of a “formulated” (well characterized) preparation and relate the needed exposure per the in-vitro model(s) (EC50)
  - Determine the PK parameter estimates and conditions of elimination (ADME, if possible) in the selected species for the ARS indication – Use the PK to select the dose → exposure
  - Relate potential indicating biomarkers to the proposed efficacious physiologic changes
  - Create a tightly bounded ILP in the species of interest
  - Establish drug efficacy in a well powered study
  - Complete all toxicology and CMC requirements per FDA consult
  - Submit IND, establish clinical safety and a useful TI from Ph 1 and Ph2 studies, and
  - Complete a pivotal efficacy per Animal Rule at the required excess exposure per the Animal Rule Guidance
What Have We Learned

• The PHEMCE partners are ready to support you in the development of your MCMs

• Drug/Biologic Development needs to satisfy TRL guidelines
  — Every drug/biologic will be different – the approaches, however, will be consistent

• Radiation dosimetry is a key fundamental that must be documented
  — The Institutional Lethality Profile is your “bedrock” for establishing reproducibility
  — The ILP need not be powered highly – the key: linearity in the operational range

• Study power matters - The role of power is to avoid 1-β errors (use the Table!)
  — Typically, a reproducible 30% improvement in survival requires an “N” of at least 31 per group
  — Equates to a power of 0.7 and an alpha of 0.025

• The EC50 translation to the effective in-vivo PK exposure is often missed
  — The exposure (drug conc vs time) must be related across two species (Guidance)
  — The MTD (max tolerated dose) and the MEC (minimum effective concentration) are important considerations for the Therapeutic Index
    • BARDA seeks CONOPs therapeutic Index (TI) values of >5 (negotiable)
    • Per The Animal Rule PK:
      — population human exposure MUST exceed the efficacious animal exposure

What Have We Learned

• The Natural Hx of an ARS injury should be measurable and consistent
  — The MCM should alter these markers in a consistent and reproducible manner toward the naïve state

• Biodistribution is a critical measure often overlooked
  — Includes the subcutaneous and oral “F” assessments and dose correction for the “F”
  — 2nd messenger PK and PD are often as important as the initiating MCM/drug itself
  — Metabolism is a key as the byproducts/metabolites may induce toxicities/ changes

• Hematopoietic, Gastrointestinal, Pulmonary and Cutaneous MCMs need to have their own respective animal models with measurable endpoints
  — TBI may not be an effective model; Use PBI when needed and appropriate
  — Natural Hx is critical and survival, while a required measure, needs supportive biomarkers
  — Supportive care is a measured need – BARDA expects early decisions under scarce resources
  — Animal models will need to be reproducible (remember ILP) and species/strain as well as biomarker technologies need to be transferable.

• The PHEMCE fulfills MCM requirements using open competition and innovation
  — The Strategic National Stockpile (CDC SNS) is one way of meeting the requirements
  — Options include: Vendor managed inventories (VMI) and other innovative plans
The Unthinkable – We think it every day

• The Unthinkable is Always Possible – We Must Be Prepared

NKOREA APPROVES 'MERCILESS' NUKE ATTACK ON USA

OBAMA TOP WORRY: NUKE BOMB NYC

NUCLEAR WAR 'UNAVOIDABLE'

Thank You!

- I wish to acknowledge the CRN staff at BARDA and at NIAID for their input to this presentation and for their continued support of the MCM development efforts in the USG.

- Thanks to the ASPR, FDA, DHS, the CDC, DoD/AFRI and other PHEMCE partners, our contracts and grants offices (AMCG), the OGC, and many more, for continued extraordinary cooperation and outstanding integration of the PHEMCE to achieve the USG goals.

- I wish to thank all the PHEMCE partner contractors in the audience, and those in the past, for your participation where we have all contributed to the understanding of this science and how to make your particular product development a success.

- Certainly a “Thank you” to the session organizers for inviting me to present today.

- And … always remember ….. ”think outside the box”!
• **Advanced Characterization of Candidate and Initiation of GMP Process Development**
  • Continue non-GLP *in vivo* studies, and animal model and assay development. Establish draft Target Product Profiles. Develop a scalable and reproducible manufacturing process amenable to GMP.

• **Animal Models**: Continue development of *animal models for efficacy* and dose-ranging studies.

• **Assays**: Initiate development of *in-process assays and analytical methods* for product characterization and release, including assessments of potency, purity, identity, strength, sterility, and quality as appropriate.

• **Manufacturing**: Initiate process development for *small-scale manufacturing* amenable to GMP.

• **Target Product Profile**: Draft preliminary *Target Product Profile*. Questions of shelf life, storage conditions, and packaging should be considered to ensure that anticipated use of the product is consistent with the intended use for which approval will be sought from FDA.

• **5A** Demonstrate acceptable *Absorption, Distribution, Metabolism and Elimination (ADME)* characteristics and/or immune responses in non-GLP animal studies as necessary for IND filing.

• **5B** Continue establishing *correlates of protection, endpoints, and/or surrogate markers for efficacy* for use in future GLP studies in animal models. Identify minimally effective dose to facilitate determination of "humanized" dose once clinical data are obtained.

*ASPR: Resilient People. Healthy Communities. A Nation Prepared.*
A study shall be adequately powered to assure the study outcome is real.

**WHAT does a value of Statistical Power mean?**

Ideally, power should be set around 0.80 (Cohen, 1988)
Pharmacokinetics

- The introduction of a drug into the body sets the stage for serial processes of absorption, distribution and elimination which are defined by PK parameter estimates, i.e.:
  - The clearance of a drug is defined through the PK terminal elimination
  - The volume of distribution defines the extent of drug distribution
  - The T1/2 defines the rate of loss of drug exposure

Parameter Estimates of importance:

\[
\text{Cl} = \frac{\text{dosage (mg/kg)}}{\text{AUC (mg/L x hr)}}
\]

\[
V_d = \frac{\text{Dose}}{C_{p_0}}
\]

\[
T_{1/2} = \frac{(0.693 \times V_d)}{\text{Cl}}
\]

\[
k = \frac{0.693}{T_{1/2}}
\]

\[
\text{Cl}_E = k \times V_d
\]

Therapeutic Index:

\[
\text{TI} = \frac{\text{Toxicity}}{\text{Efficacy}}
\]

*The larger the better*