Division of Genetic and Molecular Toxicology (DGMT)

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Deputy Director (Acting)

Division Staff

- **Government Positions – 27 full time employees (FTE)**
  - Research Scientists – 8 FTE
  - Staff Fellows – 8 FTE (2 externally supported)
  - Support Scientists- 9 FTE
  - Administrative – 2 FTE
  - FDA Commissioner Fellows – 1 staff member
  - ORISE Post Docs – 8 (2 externally supported) staff members

  TOTAL Positions = 36

- **Staff Changes from 2015 to 2016**

  (+1 permanent FTE, +3 staff fellows, +3 post docs)
  - 1 Commissioner's Fellow converted to Staff Fellow
  - 1 ORISE Post Doc externally funded for 1 year on Staff Fellow appointment
  - 1 Deputy Division Director appointment in process
  - 2 Staff Fellows in process of being converted to permanent employees

Outreach/Local

- DGMT scientists in collaboration with the University of Arkansas Medical Sciences are developing a human reticulocyte *PIG-A* assay for use in monitoring gene mutation in cancer patients receiving platinum-based antineoplastic therapy.
- As part of a Memorandum of Understanding between the State of Arkansas and FDA, division scientists perform research on the genotoxicity of the nanomaterial, graphene, in collaboration with University of Arkansas at Little Rock.

Outreach/FDA Centers

- Respond to Agency needs for chemical-specific data.
- Collaborative projects with CFSAN, CDER, CDRH, CTP.
- Performed studies on the genetic toxicity of nanoparticles, botanicals, drug impurities, tobacco products ─ used/adapted standard assays and performed mechanistic studies.

Outreach/Global

DGMT members:

- Led International Workshop on Genotoxicity Testing (IWGT) team of industry, academic, and regulatory scientists to develop a consensus report on the state of in vivo Pig-a assay development.
- Leading Health and Environmental Sciences Institute (HESI) team to validate and develop OECD TG for the assay (2015-2022).

Outreach/Global

- Development and validation of regulatory tests.
- DGMT scientists are members of Organization for Economic Cooperation and Development (OECD) workgroups:
  - Nanomaterial testing
  - Revision of existing OECD Test Guidelines (TGs) … led effort on revising *in vitro* Hprt gene mutation guideline (TG476)

DGMT Mission (Vision)

Mission:

- Improve public health by providing the Agency with the expertise and tools necessary for comprehensive assessment of genetic risk and by strengthening approaches to integrate knowledge of genetic risk into regulatory decision making.

Research Goals:

- Respond to Agency needs for chemical-specific data (e.g. nanomaterials and tobacco products).
- Maintain DGMT's tradition of leadership in regulatory assay development and validation (e.g., MLA, Hprt, **TGR, Pig-a**).
- Establish new paradigms for regulatory decision making that integrate measures of genetic risk with biomarkers of toxicity.

DGMT Research Strategies

- Engage FDA product centers, NIEHS/National Toxicology Program, and other national and international organizations to set research priorities.
- Develop better biological models for assessing human risk.
- Develop more comprehensive approaches for monitoring genetic variation.
- Develop better ways of evaluating data to determine human risk.

Three Top Accomplishments

1. Received approval from Organization for Economic Co-operation and Development (OECD) to develop and validate an OECD test guideline for the rodent *Pig-a* gene mutation assay for regulatory genotoxicity safety assessments.
2. Conducted an Office of Women's Health-funded project comparing the oncomutation profile of breast cancers in Caucasian and African-American women.
3. Developed a new transgenic hairless-albino mouse model for potential reduction of animals used for NTP photocarcinogenicity study.

Accomplishment #1
Principle of the *Pig-A* assay

- *Pig-a* = phosphatidylinositol glycan class A gene
- Gene product is required in the first step of glycosylphosphatidylinositol (GPI) anchor synthesis
  - GPI anchors attach several proteins to the surface of mammalian cells (including RBCs, e.g., CD59 and CD24)
- Of the genes required to form GPI anchors, only *Pig-a* is located on the X-chromosome
  - Meaning “one hit” can produce a cell surface phenotype
  - Resulting phenotype can be assessed with flow cytometry

Wild-type

Mutant Phenotype

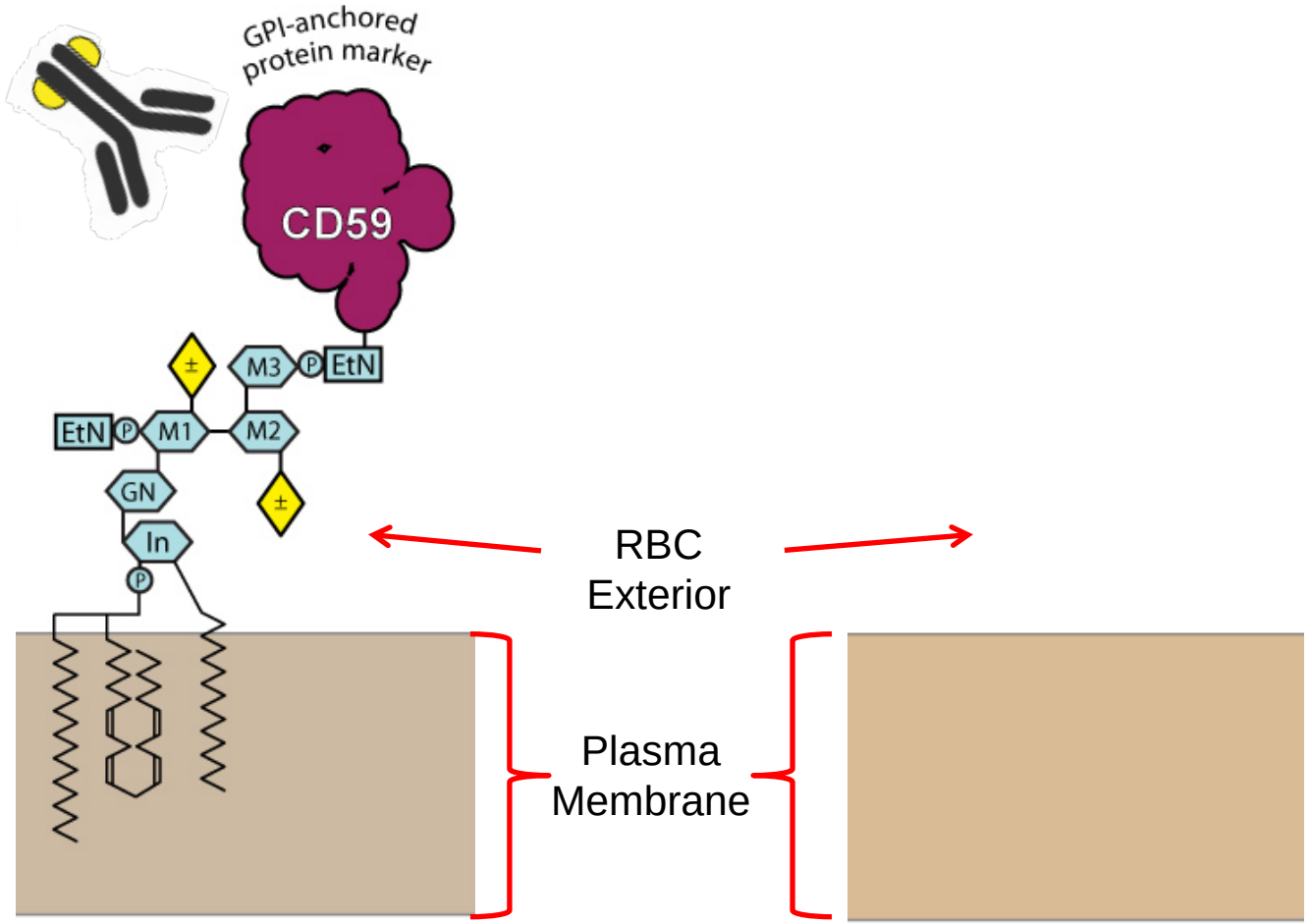


Figure adapted from **Dobrovolsky *et al.*, Environ Mol Mutagen, v51, 2010**

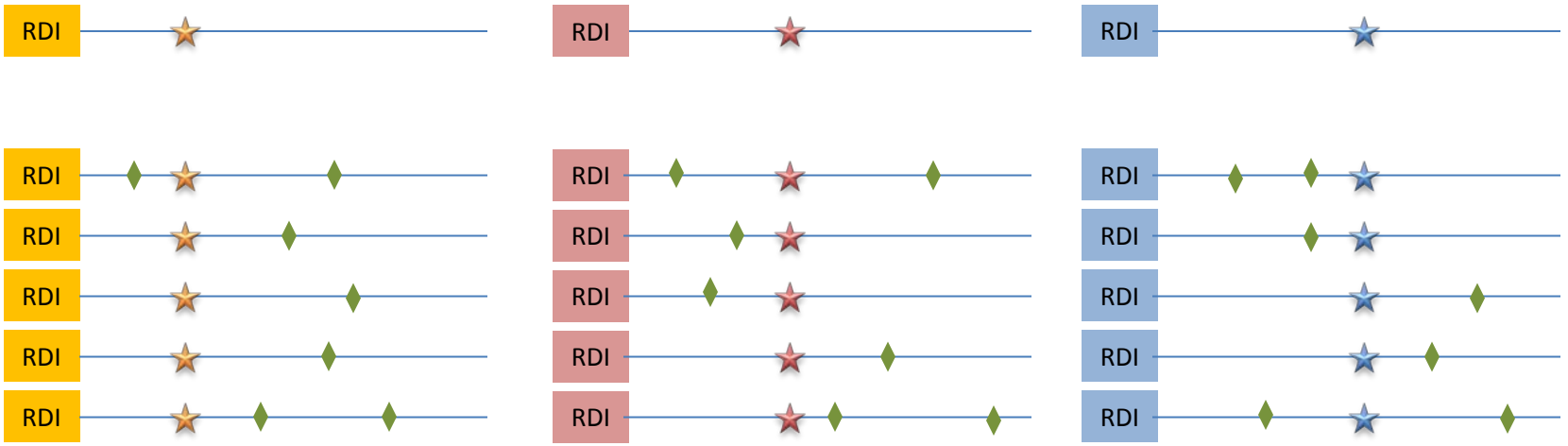
Analysis of *Pig-a* Mutant Cells by NGS

- NGS is not very accurate at the level of individual reads
- Depth of coverage is insufficient when analyzing MANY (100/1000) different mutants

Revollo et al., (2016) Environ Mol Mutagen 57:114-24

MARDI - Mutational Analysis by Random DNA Identifiers

cDNA sequencing of DMBA induced *Pig-a* mutant T-cells



MARDI: same DMBA spectrum of mutations as with Sanger sequencing, more mutations were found as many more mutants were analyzed

Highlights on the Road to Regulatory Acceptance

- 2008: first publications (co-developed at NCTR)
- 2009: adopted by ILSI-HESI for development
- 2011: magnetic enrichment introduced
- 2013: IWGT workgroup report
- 2014: M7 guidance compliance for impurity qualification
- 2015: included in the OECD WNT work plan
- 2016-17: Research at NCTR on demonstrating *Pig-a* mutations are responsible for the assay phenotype
- 2018: Detailed review paper and validation report approved
- 2021: OECD TG acceptance

Accomplishment #2

Barbara Parsons, Meagan Myers

- Characterized tissue-specific properties of ultra low frequency cancer-driver mutations (CDMs) in normal tissues (tissue-specific variability, impact of age and gender) using ACB-PCR.
- Established the prevalence of subclonal PIK3CA and KRAS mutations in breast, colon, lung, and thyroid cancers – drivers of therapeutic resistance.
- Ongoing work:
  - Characterizing subpopulations carrying hotspot somatic mutations with respect to breast cancer subtype and ethnicity (Supported by Office of Women's Health).
  - Protocols in place to characterize batteries of hotspot CDMs by error-corrected NGS and ddPCR.

Accomplishment #2

- Myers et al. (2015) Low-frequency *KRAS* mutations are prevalent in lung adenocarcinomas. *Personalized Medicine.*12:83-98.
- Myers et al. (2016) Breast cancer heterogeneity examined by high-sensitivity quantification of *PIK3CA, KRAS, HRAS, and BRAF* mutations in normal breast and ductal carcinomas. *Neoplasia.*18:253-263.

Accomplishment #3

Breeding SKH-1 mice with C57BL6 *Gpt* delta mice

***Gpt* delta mouse**
Homozygous

(*gpt/Spi*⁻ *transgenes*)

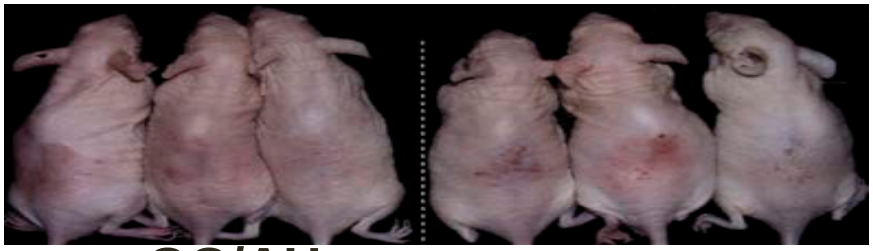
SKH-Mouse

Genotype (RT-PCR), Cull nulls & low gene dosage

11-12 wk/litter
10 pups/litter
2 litters
6 months

Transgenic, albino, hairless mice (THA)

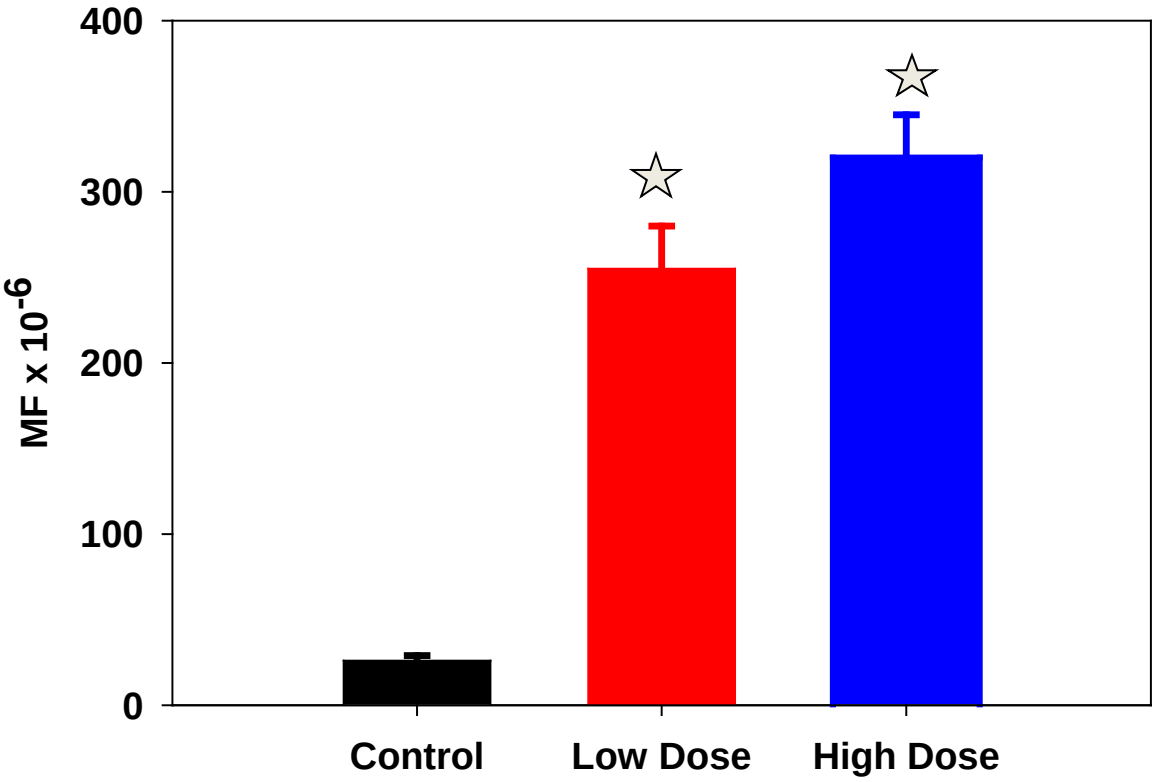
11-12 wk/litter
10 pups/litter
2 litters
6 months



GG/AH **GG/AH**

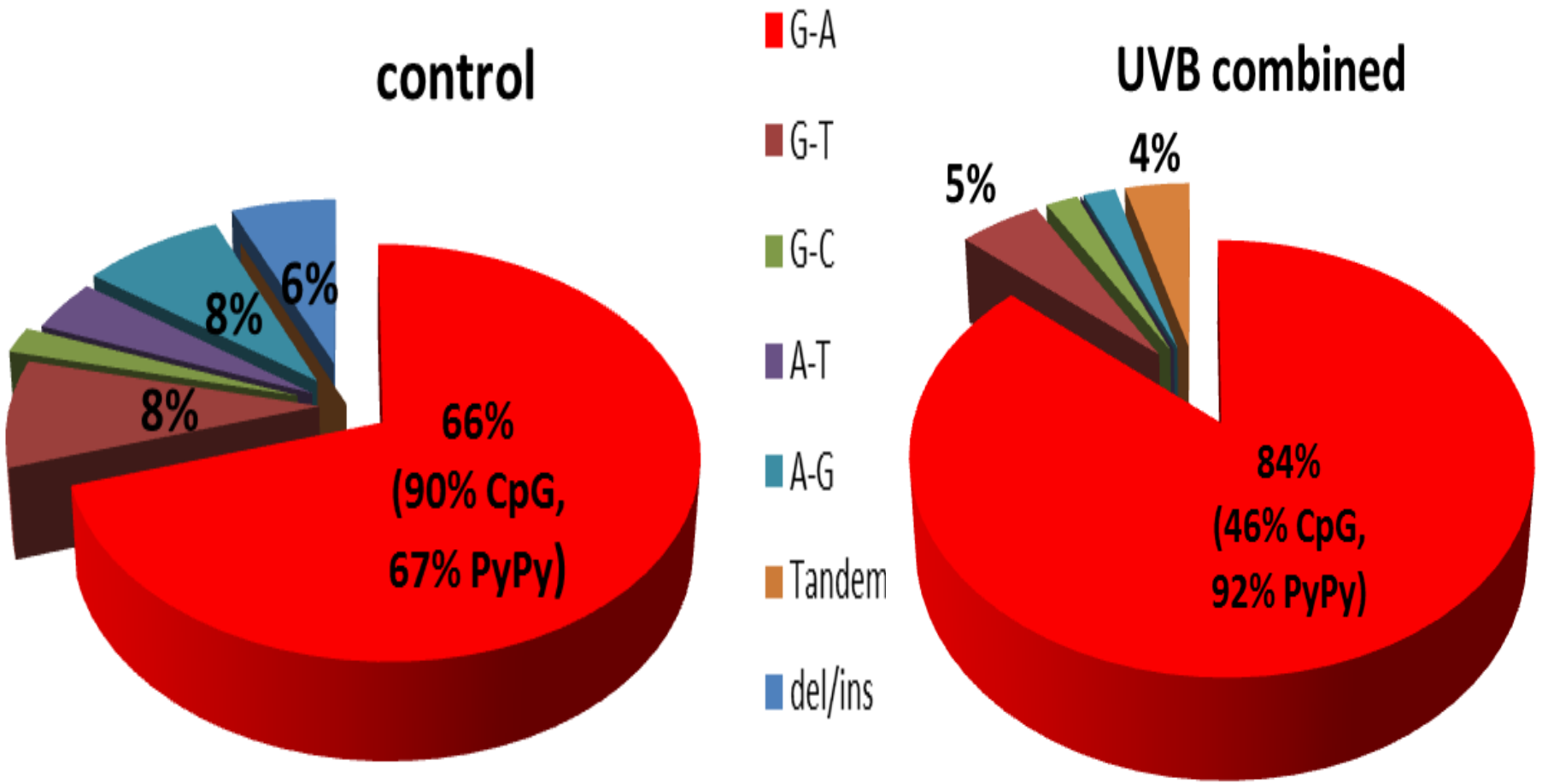
Accomplishment 3

Gpt MF in the epidermis following exposure to UVB



Accomplishment # 3

DNA sequence from *gpt* mutants
(47 from Control and 121 from UVB)



Future Areas of Emphasis

- Chemical-specific data
- 'Conventional' genetoxicology
  - gene editing (CRISPR), autophagy projects
- Developing new biological and analytical approaches
  - error-corrected NGS, ddPCR, human *in vitro* organotypic cultures, MPS
- Developing new approaches to using genetoxicology data

Future Direction/Strategy

Instead of a one-size-fits-all standard genetox battery and using genetoxicology data in a yes/no manner to identify carcinogens:

- Consider mutation as a true toxicological endpoint.
- Consider both somatic cell and germ cell mutations as key events (apical endpoints?) for human disease, not just cancer.
- Consider mutation in an integrated fashion with other toxicological endpoints, perhaps in the context of adverse outcome pathways.
- Consider mutation and the shape of the dose-response curve in a quantitative manner to evaluate risk.

Feedback Requested

- What emerging sciences/technologies can you advise me to pursue?

- What future directions do you recommend for this division that would impact the FDA?