GAME TIME: who wants to be a pathologist?

Real Life /Case based
IMMUNODEFICIENT CHALLENGES

What is it?
Who dunnit?

SBCAL 2018 rev cbrayton@jhmi.edu

3 Which mouse A or B is a better candidate recipient for a xenograft? _____

<table>
<thead>
<tr>
<th></th>
<th>A. Crl:CD1-Foxn1nu</th>
<th>B. BALB/cAn-Foxn1nuNcr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
<td>Age</td>
<td>6w</td>
<td>6m</td>
</tr>
<tr>
<td>WBC</td>
<td>3x10^3/ul</td>
<td>6x10^3/ul</td>
</tr>
<tr>
<td>TP</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Alb</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

a) Why? ____________________________________________

b) Which is out bred?

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4 62645 nu/nu
Diagnosis/es?

5 Diagnosis/es?
Likely background strain?

A. Dystrophic cardiac calcification
B. Epicardial mineralization
C. Fat necrosis
D. BALB/c
E. C57BL/6
F. NMRI

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6 NOD.CB17-Prkdcscid/SzJ, with pale eyes and paws. It is clinically dyspneic.
What is the most likely diagnosis? _______

A. Myeloid leukemia
B. Histiocytic Sarcoma
C. Plasma Cell Tumor
D. B cell Lymphoma
E. Thymic Lymphoma

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mouse retroviruses as infectious agents

A. Hartley & al. 2008
- RAW264.7 cells....
- Common mouse macrophage cell line (ATCC TIB71)
- Newborn mice developed lymphoma following inoculation

B. Thymic T cell lymphoblastic lymphoma

5 Mouse Tumors

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Sex</th>
<th>Strain predisposition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematopoietic Lymphoma, HS</td>
<td>F&gt;M</td>
<td>AKR, C58, NODscid, S/Jl/J (C57BL, etc)</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td>A (FVB, 129, etc)</td>
</tr>
<tr>
<td>Mammary</td>
<td>F&gt;&gt;M</td>
<td>C3H (BALB etc)</td>
</tr>
<tr>
<td>Liver</td>
<td>M&gt;&gt;F</td>
<td>C3H, CBA, (B6C3 ...)</td>
</tr>
<tr>
<td>Pituitary</td>
<td>F&gt;M</td>
<td></td>
</tr>
</tbody>
</table>

A. What strains are famous for what tumor?
   - Could that be useful to your tumor model?
B. Is your favorite strain tumor resistant?

10 Tg MMTV ERbB2

A. Pneumonia
B. Abscesses
C. MMTV erbB3 metastasis/embolism
D. Lung adenoma
E. Lung carcinoma

11 Tg MMTV ERbB2

A. Pneumonia
B. Abscesses
C. MMTV erbB3 metastasis/embolism
D. Lung adenoma
E. Lung carcinoma

13 NSG

Diagnosis/es:
1. Lung
   - Cause?
2. Mammary gl
3. Uterus tumor
4. Uterus +PAS
5. Uterus tumor
   - Chromogranin A
6. Lymph Node

Santagostino et al. 2017
http://journals.sagepub.com/doi/full/10.1177/0300985817698210

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14 Mammary Tumors
Xenograft Hu → Mouse
A. Foxn1<sup>nu/nu</sup> hypothymic mice lack T cells
   – Not the only hairless mice...
B. Ectopic ?
C. Orthotopic ?

15 Bone, marrow – what happened to my MCF7 mice?

MDA 468 😊
MCF7 (+ estrogen) 😊

16 A few years later...
scid – Which is (more) normal

A. Diagnosis/es?

17 Sternum
NODscid – Which is more 'normal'

Diagnosis/es?

18 Sternum
NSG 70183

Causes?
• Irradiation?
• Cytoxan ?
Susceptible strains?
1. Scid
2. Balb
3. NOD
4. B6

19 Granulocytic leukemia ?

CBC
A. WBC = 23000!
B. Ne = 13000
C. Ly = 6000
   – Mono & LUC
   – r/o blasts
   – Evaluate smear

MARROW →
20 Lung
- 10moF
- Breeder
- Tm?
- Swollen face
  A. Leukemia?
  B. Infection?

21 Lung
- 10moF
- Breeder
- Tm?
- Swollen face
  A. WBC > 100,000
  B. IV leukocytosis

22 Lung
- 61998
- Quarantine
  A. Leukemia?
  B. Infection?

23 Tissue?
  A. Which is most ‘normal’
  B. Which is from an Athymic mouse?

24 Dying Mice
- NODscid Mortality post irradiation
  A. CAUSE?

25 Dying mice – 8-10 w post irradiation
  A. Tissue?
  B. Which is abnormal
26. Dying mice – 8-10 w post irradiation
   A. Tissue?
   B. Which is abnormal

Beware of irradiation etc genotoxic damage on growing cells (hypodont teeth)

27. C57BL/6 NOS
   A. Which is normal?
   B. Diagnosis/es?
   C. Causes?
   D. Human relevance?

A. Enamel hypomineralization
   amoxicillin/clavulanic acid Rx
   Amoxicillin Rx in early childhood may be associated with enamel hypomineralization

Mihalaș et al. 2015
http://journals.sagepub.com/doi/abs/10.1177/0192623315610822

28. Antimicrobials – other effects

Clavamox
- Dysfunction in maturation and transitional ameloblasts, resulting in hypomineralized enamel

OTHERS:
- Enrofloxacin
- Cartilage effects in growing animals
- Trimethoprim Sulfamethoxizole
- Thyroid effects
- Fenbendazole
- Immunomodulatory
- Anticancer?

Mihalaș et al. 2015
http://journals.sagepub.com/doi/abs/10.1177/0192623315610822

29. Post Xenograft Survival

A. Post irradiation + 6m Bactrim Rx.
B. Could this cause a clinical problem?

SPORTS

MOUSE: Etiology?

Likely mouse strain?

Don’t panic yet...
Are these BALB/c or SJL/J males?

Dying mice – 8-10 w post irradiation
A. Tissue?
B. Which is abnormal

Beware of irradiation etc genotoxic damage on growing cells (hypodont teeth)

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SPORTS

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Are these BALB/c or SJL/J males?
32 Ectromelia Virus
A. Orthopoxvirus, causes mousepox
   - Species specific orthopoxvirus

B. WAS a cause of devastating 'outbreaks'
   - Variation in mouse strain susceptibility

C. In biological materials near you!!
   - 'outbreaks' from commercial mouse serum....

33 C57BL/6J
A. Diagnosis/es
   - Liver necrotizing multifocal
   - Splenomegaly

B. Cause/s?
   - Administered biologicals ?
   - Immunodeficiency X
   A. Ectromelia virus
   B. Mouse Hepatitis virus
   C. Murine Cytomegalovirus
   D. Murine Norovirus
   E. Clostridium piliforme
   F. Helicobacter hepaticus
   G. Salmonella e. typhimurium

35 C57BL/6J
A. Diagnosis/es
   - Liver necrosis multifocal
   - Splenomegaly
   - necrosis multifocal

B. Cause/s?
   - Administered biologicals
   A. Ectromelia virus
   B. Mouse Hepatitis virus
   C. Murine Cytomegalovirus
   D. Murine Norovirus
   E. Clostridium piliforme
   F. Helicobacter hepaticus
   G. Salmonella e. typhimurium

36 A. Tissue?

B. Which is from a 12g 6mo scid mouse?

C. Which is from a BALB/c mouse?

D. What is the cause? (agent)

37 Sendai V
Inflammation in competent mice

B. Proliferation/healing in survivors
38 64609
A. 14dpi Pr8 – IL10 ko survivor

39 64599 64604 C57BL/6+PR8
A. Most B6 die by ~9dpi PR8
B. IL10 KO survive longer

40 A scid mouse received a tumor cell line ....
A. Tissue?
B. Lesion?
C. Cause?

42 Renal INIB dt??

43 Santagostino et al 2017: NSG
A. 2 INIB in tubule epithelium
   - Large amphophilic
   - Small eosinophilic
B. Karyomegaly, tubule degenerative necrosis
C. TEM e-lucent schmutz
D. Serology, PCR, IHC negative for viruses
   - 1 report of positive IHC for a bovine papillomavirus antigen
E. Cohoused immune deficient develop similar lesions
   - CD-1 do not

44 Roediger et al in press
→ Special Stains?

Cause(s)?
A. Adenovirus?
B. Herpesvirus?
C. Parvovirus?
D. Polyomavirus?
E. Other?
F. IMMUNODEFICIENT
→ Other tests?
47 What might be more interesting?

A. BK virus
B. JC virus
C. SV40
D. Rat PyV2
E. MAd1 Fl
F. Mad 2 K87
G. What should stain these INIB?

48 Pathogenic DNA Viruses

<table>
<thead>
<tr>
<th>FAMILY</th>
<th>VIRION STRUCTURE AND SIZE</th>
<th>GENOME STRUCTURE AND MOLECULAR WEIGHT</th>
<th>REPRESENTATIVE MEMBERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poxviridae (Poxviruses)</td>
<td>icosahedral, naked 20 nm</td>
<td>ds linear (1.5–2.5×10^9)</td>
<td>Human papillomavirus 6-18; adenovirus-associated virus</td>
</tr>
<tr>
<td>Hepadnaviridae (Hepadnaviruses)</td>
<td>icosahedral, enveloped 42 nm</td>
<td>ds linear (2–2.5×10^7)</td>
<td>Hepatitis B virus of humans, woodchuck hepatitis virus</td>
</tr>
<tr>
<td>Polyomaviridae (Polyomaviruses)</td>
<td>icosahedral, naked 45 nm</td>
<td>ds circular (2–2.5×10^7)</td>
<td>Papilloma virus, BP-1virus, WPV-1virus, Merkel cell virus, HPV-7 of humans, SV40 (monkey)</td>
</tr>
<tr>
<td>Adenoviridae (Adenoviruses)</td>
<td>icosahedral, naked 60-110 nm</td>
<td>ds linear (1.5–2×10^11)</td>
<td>Human adenoviruses 5 and 7, human papillomaviruses 6 and 7, human herpesviruses 5 and 7</td>
</tr>
<tr>
<td>Herpesviridae (Herpesviruses)</td>
<td>icosahedral, naked 180-200 nm</td>
<td>ds linear (1.5–2×10^11)</td>
<td>Human herpesviruses 1, 2, 4, 5, 6, 7, 8 and 10; Epstein-Barr virus, human papillomavirus 5 and 8; human cytomegalovirus, human adenovirus 5; herpes simplex virus types 1 and 2; varicella-zoster virus; cytomegalovirus; Epstein-Barr virus; human herpesviruses 5 and 7, human herpesvirus 5</td>
</tr>
</tbody>
</table>

A. Papovaviruses & Adenoviruses exist as naked unenveloped nucleocapsids
B. Papovaviruses tend to have a random, rather than straight line arrangement of individual virions; the converse is true for adenovirus, which is usually in paracrystalline arrays.


A. Parotid
B. Bronchiole
C. Prostate
D. Harderian

CAUSE?
• RatPyV2

54 NSG

Diagnosis/es:
7. Skin + PAS
   • Cause?
8. Kidney
9. Kidney EM
   • Cause?
10. Head (decal)
11. Spine
12. Kidney

Santagostino et al. 2017
http://journals.sagepub.com/doi/full/10.1177/0300985817698210
Copyright © 2017 McGraw-Hill Education. All rights reserved
Sherris Medical Microbiology, 6e
Available at: http://accessmedicine.mhmedical.com/Content.aspx?bookId=1020&sectionId=56968657
Accessed: November 09, 2017
PDX: carcinoma GREAT TUMOR TAKE.... 😊

Primary tumor (human)  PDX spleen

PDX ‘carcinoma’ recipient
Excellent ‘take’?

A. Human carcinoma
B. PDX orthotopic + skin → excellent take + splenomegaly
C. Telomerase confirms human

EBV Lymphoma in (PDX or humanized mice)


Bronchopneumonia (+ AMP)

B6.129P2-Il10tm1Cgn/J (Il10 KO) Il10 knockout

A. Diagnosis /es
B. Cause(s)
   • Lobar consolidation with bronchitis, bronchopneumonia
   • Also acidophilic macrophage pneumonia
      – P pneumotropica?
      – B hinzii, avium?
      – Klebsiella oxytoca?
      – K pneumoniae?

human xenograft p1 kidney
DNA (DAPI)
Tissue Background

57
A. Human yellow arrowheads
B. Mouse white arrows
C. A Meeker

58

59

18 SBCAL Brayton 1 Immunodef GAME :)

62. Nose, Gall bladder Diagnosis/es? What is being produced?
   A. Inflammation, proliferation, hyalnosis
   B. YM1 or YM2 chitinase like protein

63. B6.129P2-Il10<sup>tm1Cgn</sup>/J (Il10 KO)
   UPPER Respiratory infection: Tracheitis
   A. Who dunnit?
      – P pneumotropica
      – B hinzii, avium?
      – Klebsiella (oxytoca)?
        • Immunodeficient?
      – Mycoplasma?
        • Not so common
      – Streptococci?

64. B6.129P2-Il10<sup>tm1Cgn</sup>/J (Il10 KO)
   UPPER Respiratory infection: Rhinitis
   A. Mice are obligate nose breathers
   B. This could kill them
   C. Who dunnit?
      – P pneumotropica
      – B hinzii, avium?
      – Klebsiella (oxytoca)?
        • Immunodeficient?
      – Mycoplasma?
        • Not so common
      – Streptococci?

65. B6.129P2-Il10<sup>tm1Cgn</sup>/J (Il10 KO)
   UPPER Respiratory infection: Otitis
   A. Pretty common in our mouse submissions
   B. Effects on
      – Hearing?
      – Behavior?
      – Immune responses?

66. Cause? Etiology?
   A. Gram negative Bacilli adhere to cilia
   B. Hard to identify on histo try a silver stain
   SEM of B bronchiseptica on cultured rabbit tracheal epithelium
   Edwards & al 2005
LAD (leukocyte Adhesion Deficiency) CD11/18 deficient

A. Inner ear?
B. Middle ear?
C. Bulla
D. Ear drum (tympanum)
E. External ear
   Ear canal
F. Diagnoses?
G. How to get histo?

Otitis

Opportunists? Or Pathogens?
- *P. pneumotropica*?
- *K. oxytoca*?
- *B. hinzii/avium*?
- *Mycoplasma*?
  - Historical?
- *Pseudomonas*?
  - Historical?
  - Only? in immunodeficient?
  - Neutrophil deficient

Aural Cholesteatoma (chronic otitis)

59113 4mo F

Otitis – fairly common, but...
When its this bad – are they immunodeficient?

Gram+
- Strep/Enterococcus

Gram-
- Pasteurella Bordetella

Gram 0
- ?
- Mycoplasma

Rolling, Spinning, Head tilt
Diagnosis /es?

A. GEM Model of vestibular syndrome?
B. Otitis?
C. Arteritis?
D. Tumors?
E. Other?
74 Malacia, necrosis compatible with ischemia/infarct
- Southard & al 2007, 2
- Acute onset spinning
- → death
- >12 mice of 2 Swiss stocks; 1 source – local

75 6318 ~6mo CD1 F sentinel
Found Rolling

76 54530 adult? Genotype ???
found dead AFTER quarantine

77 Gram stain (otitis)
A. Gram positive cocci
B. Pairs (diplococci)
C. Chains (streptococci)
Enterococcus?
Streptococcus?

78 K oxytoca otitis in C3H/HeJ
Macarthur & al 2007

Note ‘capsules’

79 Experimental infection
A. What could this be in a susceptible mouse?
80  B6 co housed with pet store mice....

A. Diagnosis/es?
B. Cause/s?
C. Etiologic diagnosis/es?

Cornax & Osullivan 2017
LCPG & V Carriera
http://www.cldavis.org/diagnostic_exercises.html

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81  B6 co housed with pet store mice....

A. Diagnosis/es?
B. Cause/s?
C. Etiologic diagnosis/es?

Cornax & Osullivan 2017
LCPG & V Carriera
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82  Infertility Pyometra

A. Likely Causes?
   - P pneumotropica?
   - M pulmonis?
   - Imperforate vagina
     • Vaginal septa

B. Opportunists in immuno-deficient immunoweird

85  MOUSE: Morphologic Diagnoses. What are possible-likely causes? (Slide 1 of 2)
MOUSE: Morphologic Diagnoses. What are possible-likely causes? (Slide 2 of 2)

Lethal Liver necrosis in immunodeficient mice...
A. *Clostridium piliforme* –
B. also
   – MHV
   – (MCMV) Cytomegalovirus
   – ECT Ectromelia virus
     • (Spleen necrosis, inclusions, ectromelia)
   – Helicobacters?
   – *Salmonella enterica typhimurium*

Abscesses
A. S aureus or
B. Coagulase NEG
   Staphylococcus spp
   e.g. S xylosus, S hyicus etc. in immunodeficient or immunoweird
C. Botryomycosis

Salmonella enteritidis...
A. *S typhimurium* -- RADIL

Mouse / GEM Diagnosis?
A. Neoplasia (head/neck tumor e.g. SCC Squamous Cell Carcinoma )
B. Abscesses (e.g. Botryomycosis )

Mortality in Myd88 KO
Brain
A. Gram stain
B. Overwhelming gram positive bacteremia and bacterial colonization
C. *E faecalis* isolated from some
96 Myd88 KO
A. Gram negative infection
B. likely cause?
C. TREATMENT?
D. PREVENTION??

(myeloid differentiation factor 88 deficient – no innate immunity )

97 ☺ Mortality NSG Post Xenograft ☺

Reports of
Klebsiella oxytoca
Enterococcus fecalis

NSG UTI urinary tract infections & sepsis – Foreman & al 2010

98 ☺ Mortality NSG Post Xenograft ☺
Kidney, gram stain

Gram negative rod
– Capsule ?
A. Klebsiella oxytoca ?
B. E coli ?

101 GEM w surfactant defect
Model of Pulmonary Alveolar Proteinosis?

103 Immunodeficient mice (MMAD)
Found dead or Respiratory disease noted.

• Consolidation
• Intra-alveolar eosinophilic foamy material
• o-Minimal inflammation

A. Special stain ?
B. Other diagnostic tests ?
104 Immunodeficient mice (MMAD)
Found dead or Respiratory disease noted.
A. Sudden Death
B. Diffuse consolidation
C. interstitial pneumonia

105 Acidophilic Macrophage Pneumonia
Eosinophilic Crystalline Pneumonia
A. Murray & Luz 1990
B. Granulocyte / Charcot Leyden material →
C. YM1 Chitinase like Protein →

106 Acidophilic Macrophage Pneumonia
Eosinophilic Crystalline Pneumonia
A. 1990 (Murray & Luz) → Granulocyte / Charcot Leyden material →
B. 1990 (Guo&al.) YM1 Chitinase like Protein →

107 HUMAN PAP: EM lamellar bodies in alveoli

108 HUMAN PAP: Few constipated macrophages

110 65286 - 6
A. Tissue?
B. Diagnosos/es?
111 e.g. Degu stomach
A. PAS positive Easter eggs

112 Gastric Yeasts
   – Torulopsis pintolopesii - ON surface

115 Mouse Stomach: WYD?

116 Mouse Stomach: WYD?

117 WYD?
A. From an anonymous source via J Ward
Cryptosporidia in mice

Stomach (glands) Ileum

Tyzzer 1910


– Merozoites 5-8u
– Macrogametocyte 5x4u
– Oocysts 5-8u

Ileum: C tyzzeri (C parvum)

Fig. 4. Cryptosporidium tyzzeri in jejunum and ileum of mice under transmission electron microscopy. (A) A trophozoite in the jejunum. (B) A trophozoite in the ileum. (C) A type I meront containing eight merozoites in the jejunum

A. NOTE SIZE = TINY – oocysts ~ 4u


Nude sentinel

– Abundant in nu/nu but NOT nu/+ cagemates
– nu/+ cleared to undetectable levels
– o-minimal inflammation
– Expect these in hamsters ...

Giardia lamblia (NEJM)

Nude sentinel

– Abundant in nu/nu but NOT nu/+ cagemates
– nu/+ cleared to undetectable levels
– o-minimal inflammation
– Expect these in hamsters...
Small intestine

Historically considered to be pathogenic...
May be 0-minimal inflammation...

A. Giardia muris
- (G lamblia)
- Flying saucers
  - 'On' mucosa
  - Enteritis?
- Hamsters
- Wild mice
  - Peromyscus

B. Spironucleus muris
- (Hexamita muris)
- Torpedoes
  - In crypts
  - Enteritis?
- Hamsters
- Wild mice
  - Peromyscus

Flagellates in large intestine

A. Commensal – always?
B. Pyriform trophozoites
C. Can fill lumen of cecum, prox colon
D. Characteristic movements
  - Trichomonads (T muris, T diminuta..)
  - Hexamastix muris...
  - Chilomastix bettencourtii..
  - Cercomonas, Monocercomonoides
  - Retortomonas, Octomitus etc

Enteric Flagellates

A. Eukaryota › Diplomonadida › Hexamitidae › Giardiinae › Octomitus
B. Eukaryota › Diplomonadida › Hexamitidae › Hexamitinae › Spironucleus
C. Eukaryota › Diplomonadida › Hexamitidae › Giardiinae › Giardia
D. Eukaryota › Parabasalia › Tritrichomonadida › Tritrichomonadidae › Tritrichomonas
E. Eukaryota › Parabasalia › Honigbergiellida › Hexamastigidae › Hexamastix
F. Eukaryota › Rhizaria › Cercozoa › Cercomonadidae › Cercomonas
G. Eukaryota › Retortamonadidae › Chilomastix
H. Eukaryota › Retortamonadidae › Retortomonas

Entamoebae ~>10 µ—probably has helicobacters too
Feral mouse on campus

A. Tissue?
B. Cause? Diagnosis?

Diagnosis/es? (A-C)
What do you suspect about the mouse? (D-G)

A. Ascariasis
B. Strongyliasis
C. Oxyuriasis

D. Immune deficient
E. Immune sufficient
F. Young
G. Old

Pinworms + protozoa
Immunodeficient? or dirty?

Gravid Pinworm
Gut
Eggs
Protozoa
Flagellates
Entameeba

37572 Pet rat
A. Diagnosis/es

Rnana – Zoonotic…

Diagnosis/es?

58316 Farm Rat:

H. diminuta

Mouse: Etiology?
Rx (treatment)?

Etiology?

Rx (treatment)?

http://www.cdc.gov/parasites/hymenolepis/biology.html
Cysticercus fasciolaris (T. taeniaeformis) Where is the cat?

Common ? Arthropods
Which is:
A. A blood sucking mite?
B. A fur mite?
C. A non parasitic pest?

A. A blood sucking mite
B. Ornithonyssus Bacoti
C. A non parasitic pest

A. Ornithonyssus Bacoti
B. Ornithonyssus musculi
C. Psocid (bark or book louse)

Laelaps echidnina • Spiny rat mite
Ornithonyssus bacoti • Tropical rat mite

Diagnosis
A. NOT on mice – Eat & leave
B. 1mm moving spot on a filter top
C. Or rash on investigator

Pests NOT Parasites
Psocids
A. Book lice aka ‘Dust mites’
B. Moving critters in room
C. Not on mice
D. Like Cellulose & Humidity
E. Allergenic?
F. → hypersensitivity?

A. Human Head lice – Pediculus humanus capitis
Psocids
• Book (Bark) lice
– 6 legs + antennae
– Wings in some adults
145 WYD?

146 WYD?

Thanks! Obrigado!

A. HOSTS!! AUDIENCE!!

B. Mice & GEM
C. Nadine Forbes
D. MCP & core faculty
E. LAM & Vet path trainees on — NIH T32 RR0077022
F. Email cbrayton@jhmi.edu
G. Google Hopkins + Phenotyping

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