TSRL Microbiology and Molecular biology knowledge gaps by project

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The following identifies gaps in the data packages for projects at TSRL that would advance projects and promote the submission of grant proposals.

Priority: = High = Medium = Low

# 201-NPP

For the PhaseIIb or another broad-spectrum NPP grant:

* **Bring knowledge of NPP field up to date**:
  + **Priority and Rationale**: High for NPPs, See multi-project goals for additional rationale.
  + **Protocol**: Update the Phase IIb introduction and preliminary data sections of Research Plan with current supporting data/publications.
  + **Resources needed**: Journal access (currently through Kyle at MSU)
  + **Estimated deadline**: 3/1/19
* **Determine therapeutic window - Dose titration and dose interval for NPP-669 and NPP-671 efficacy against mCMV**:
  + **Priority and Rationale**: High Priority. We observed GI toxicity following BID oral dosing of NPPs, but NPP-669 appears to be as effective at inhibiting viral replication as the control at the lowest dose tested (10 mg/kg/day). We need to determine the lowest effective dose in order to determine the therapeutic index between the NOAEL and the lowest effective dose. If a sufficient window exists, toxicity concerns may be reduced. *If the lowest effective daily dose is toxic, this project may not be viable.*
  + **Hypothesis**: NPP candidates are effective and safe following oral administration at doses lower than reported for CMX-001 (1.25 mg/kg/day) and similar dosing intervals (QW or BIW).
  + **Protocol**: Experiments with lower doses of NPP-669 are underway and currently outsourced through NIAID as part of study 201-109. I will write the final report amendment for the current study. Efficacy studies using dosing intervals to match CMX-001 clinical dosing with NPP-669 and dose titration for NPP-671 are anticipated pending the results of the ongoing study.
  + **Resources needed**: Sufficient compound and vehicle at the study site. Non-Clinical Evaluation Agreement (NCEA) w/ attached Service Request Form (SRF) from NIAID.
    - Enough NPP-669 is available for additional studies. Less NPP-671 is available, which will restrict our ability to do multiple assays prior to a September grant submission.
  + **Estimated deadline: September grant submission**
    - The report for dose titration down to 1 mg/kg/day for NPP-669 should be completed by 3/1/19.
* **NPP-669 and NPP-671 PK and biodistribution of pro- and active drug**.
  + **Priority and Rationale**: High. The biodistribution of NPP candidates, based on the first generation candidates (USC series), appears superior to CMX-001. CMX-001 accumulates in the small intestine following oral administration, resulting in GI toxicity, while USC-505, the prior CDV prodrug lead candidate, was more evenly distributed across all tissues, but not as efficacious as CMX-001. If the prodrug is present but not converted to the active form in certain tissues, this could explain the discrepancy observed between *in vitro* potency, *in vivo* efficacy, and tissue distribution for CMX-001 and USC-505. Determining the active drug levels in tissues would allow us to better predict efficacy *in vivo* and argue for NPP superiority over oral CMX-001.
  + **Hypothesis**: NPP candidates are more evenly distributed throughout the body and more readily converted to active drug in tissues than CMX-001.
  + **Protocol**: Single-dose PK studies with animals (n=4) will be dosed using similar dosing intervals across doses ranging from 3-100 mg/kg/dose. Organs and blood will be collected to determine tissue concentrations.
  + **Resources needed**: Sufficient compound (669 and 671 with CMX-001 as a control). Animals.
    - Methods to measure NPPs from plasma and tissues using the LC-MS/MS are established, but are not yet optimized for active CDV metabolites. Further method development will be required to measure tissue levels at TSRL, using modified methods published by Ed Acosta’s group or with assistance from Jim Vrbanac. Analysis may also be available through out-sourcing via Pyxant labs (<http://www.pyxant.com/index.htm>), which appears to have been used by Chimerix for CMX-001 metabolite analysis based on the Sethna et al “intracellular conversion of BCV” poster.
  + **Estimated deadline**: **September grant submission**
* **NPP-669 and NPP-671 toxicology using longer dosing intervals**.
  + **Priority and Rationale**: High. Changing the dosing interval could reduce any adverse effects of treatment but maintain efficacy, if the PK and biodistribution of NPP candidates support longer intervals between dosing. This would allow us to argue superiority over oral CMX-001, as CMX-001 has used once and twice weekly oral dosing intervals in clinical trials and still observe signs of GI toxicity.
  + **Hypothesis**: Longer intervals between oral NPP doses will reduce GI toxicity compared to CMX-001.
  + **Protocol**: Multi-dose Toxicology studies using longer intervals between doses. Animals (n=4/group) will be dosed QW or BIW across doses ranging from 3-30 mg/kg/dose. Organs and blood will be collected for clinical and histopathology.
  + **Resources needed**: Drug and animals.
    - We have enough NPP-669 to do additional analyses, but funding may be restrictive for histology and clinical pathology.
  + **Estimated deadline**: **September grant submission**
* **Demonstrate broad-spectrum of potency and efficacy**:
  + **Priority and Rationale**: Medium. The medical need for broad-spectrum antivirals is high. We must demonstrate the NPP candidates are potent against a broad spectrum of viruses to support this claim. Sufficient potency testing has been completed for NPP-669 with efficacy for CMV, but demonstrated efficacy against additional viruses *in vivo* would strengthen our case. Additionally, more potency data is needed for NPP-671 than can be done by April, especially prior to additional efficacy testing.
  + **Hypothesis**: NPP candidates are potent against a broad spectrum of dsDNA viruses.
  + **Protocol**: These studies are outsourced through NIAID.
  + **Resources needed**: Approved SRF submissions and sufficient compound and vehicle at test sites. Costs are due to compound synthesis and shipping.
  + **Estimated deadline**: **September grant submission** for NPP-671. Largely complete for NPP-669 and CMV.
* **NPP Cytotoxicity and Intracellular Conversion**
  + **Priority and Rationale:** Medium priority. Determining the cytotoxicity and intracellular conversion of the NPPs using cell lines from different tissues would allow us to determine if specific tissues are capable of metabolizing the NPPs to CDV. Concurrently determining cytotoxicity in these cell lines would also allow us to predict which tissues would be adversely affected by treatment. We would also assess the prodrug moiety for cytotoxicity.
  + **Hypothesis**: The intracellular conversion and cytotoxicity of the NPPs are different by tissue type.
  + **Protocol**: Intracellular Conversion Assay protocols with Cytotoxicity and/or Proliferation Assays, such as the MTT assay or the CellTiter-Glo® Luminescent Cell Viability Assay, adapted for use at TSRL.
  + **Resources needed**: Cell lines from representative tissues types or primary cells from mice. Cell culture consumables and reagents. Kits for cell viability assays.
  + **Estimated deadline: September 5 grant submission**
* **Explore alternative indications for the NPPs**:
  + **Priority and Rationale**: Low. Cidofovir is potent against a broad-spectrum of viruses and NPPs may be superior against the same viral indications utilizing administration routes that avoid GI toxicity, like local or transdermal application. CDV is also effective in treating viral cancers, which may represent a large market opportunity with wider therapeutic windows for an oral CDV-prodrug chemotherapy. If we can get approval as a cancer therapy, off-label use for viral infections could lead back to NPPs as clinically viable broad-spectrum antivirals. Additionally, agricultural targets may be of value.
  + **Hypothesis**: NPPs with CDV as a warhead are effective against viral cancers, or are viable therapies in otherwise healthy humans, or animals, with dsDNA viral infections.
  + **Protocol**: Provide a report on the possible medical and commercial value of the NPPs as an oral chemotherapy, with limited toxicity, directed at cancers and skin lesions caused by dsDNA viruses by 8/1/19. Other human and animal indications (12/31/19)
  + **Resources needed**:
  + **Estimated deadline**: 12/31/19

**Publications in support of new NPP grants:**

* + **Priority and Rationale**: Publishing will help build the brand and aid in getting grant funding
* **Submit USC-505 Intracellular Conversion paper** 
  + **Estimated** **deadline**: 4/1/19
* **Draft manuscript for NPPs describing synthesis, potency, PK and distribution, efficacy, and toxicology as a “finished product” from the R03 grant and prior funding**
* **Estimated** **deadline**: 12/31/19

# **925- Zanamavir MN**

* **Bring knowledge of field up to date**
  + **Estimated deadline**: 6/1/19
* **Spectrum of ZAN Efficacy**
  + **Priority and Rationale**: Low priority. Proof of concept has been demonstrated in mice dosed subcutaneously against a pandemic, oseltamivir-resistant influenza virus (strain A/Hong Kong/2369/2009 (H1N1pdm)) in a lethal mouse infection model, especially at 100mg/kg/day QD. The Phase I grant discusses further characterization of this dosing regimen using this model against other virus strains. While this would add value for comparing transdermal (SC or MN) ZAN efficacy to other influenza therapeutics, much of these data may be found in the literature.
  + **Hypothesis**: Zan-MN (or subcutaneous Zan to mimic MN) is effective against a broad range of seasonal and pandemic flu strains.
  + **Protocol**: This work would be done through the NIAID service contract program.
  + **Resources** **needed**: ZAN, Non-Clinical Evaluation Agreement (NCEA) w/ attached Service Request Form (SRF) from NIAID
  + **Estimated** **deadline**: 1/1/2021

**Awaiting a biodegradable microneedle formulation:**

* ***In vitro* Skin Permeation** 
  + **Priority and Rationale**: High, once a lead formulation is ready. To optimize and ensure the MN array size and needle strength and number results in the required level of flux across skin. Pig skin will be used in advance of *in vivo* PK studies with minipigs. Flux across donated human skin will be determined with the fully optimized formulation.
  + **Hypothesis**: The ZAN-MN formulation will result in flux across skin greater than the target flux rate of 2.6 μg/hr.
  + **Protocol**: As described in the ZAN-MN Phase I grant and related publications.
  + **Resources** **needed**: Pig Skin, Human Skin from, ZAN-MN formulation
  + **Estimated** **deadline**: pending MN formulation
* **PK in minipig**
  + **Priority and Rationale**: Medium, once a lead formulation is ready. Ensure ZAN levels stay above the influenza IC50 following ZAN-MN administration for several days.
  + **Hypothesis**: The ZAN-MN formulation will result in flux across skin greater than the target flux rate of 2.6 μg/hr.
  + **Protocol**: Through a CRO (Sinclair), compare the PK of intravenous bolus and transdermal applications of ZAN in minipigs. Further details can be found in the ZAN-MN Phase II grant and related publications.
  + **Resources** **needed**: Animals, ZAN-MN sent to test site
  + **Estimated** **deadline**: pending MN formulation
* **Efficacy in Ferrets**
  + **Priority and Rationale**: Medium, once a lead formulation is ready. Demonstrate ZAN-MN patches are effective against flu infection in an animal model that predicts human effects.
  + **Hypothesis**: The lead formulation of ZAN-MN is effective at reducing flu symptoms.
  + **Protocol**: Through a CRO (IIT Research Institute). Further details can be found in the ZAN-MN Phase II grant and related publications.
  + **Resources** **needed**: Animals, ZAN-MN sent to test site
  + **Estimated** **deadline**: pending MN formulation

# 927 – CPP

* **Bring knowledge of field up to date**
  + **Estimated deadline**: 5/1/19
  + Maintain the Zotero Reference library continuously (ie add recently published and relevant papers as they are seen) for the project accordingly
* **MIC90 potency studies on pathogens of interest**
  + **Priority and Rationale**: High priority in support of Phase II grant in September 2019. Cell penetrating peptides represent a promising broad-spectrum therapeutic for intracellular pathogens, as they can cross the membrane of host cells and act on invading bacteria. They also show activity against biofilms. Potency screening against multiple isolates (10-15) of extra- and intracellular pathogens, especially ESKAPE pathogens, and establishing MIC90 values would verify the broad-spectrum of CPPs, as well as solidify our target indications and lead candidates.
  + **Hypo**: CPPs are effective against a broad spectrum of G+ and G- bacteria, including intracellular pathogens
  + **Protocol**: Screening done by Purdue
  + **Resources needed**: CPPs will be synthesized and tested at Purdue
  + **Estimated deadline: September grant submission**
    - MICs for multiple strains of some pathogens have been determined, but it is unclear if these experiments are equivalent in methods. If not, MIC determination will need to be repeated with a standardized protocol, otherwise testing of additional isolates to reach a final number of 10-15 will suffice for MIC90 determination
* **Cytotoxicity Evaluation**
  + **Priority and Rationale:** Lower priority for older CPP compounds. Higher for the GAP series. Determining the selectivity indices of the CPP compounds will demonstrate their potential safety. Developing protocol would also be useful for other projects where cytotoxicity data would be valuable.
  + **Hypothesis**: The GAP series of peptides will exhibit similar qualities as older CCPs in terms of cytotoxicity.
  + **Protocol**: Could be done at Purdue or TSRL.
  + **Resources needed**: Compounds, cells, culture reagents and supplies. Determining cytotoxicity and/or proliferation assays compatible with our plate reader requires further research. Possibilities include MTS assays (CellTiter 96® AQueous One Solution Cell Proliferation Assay, $326/1000 assays) or CytoTox-Fluor™ Cytotoxicity Assay ($533, 5 x 10 mL) could be used at TSRL .
  + **Estimated deadline: September 5 grant submission**
* **Resistance to CPPs**
  + **Priority and Rationale**: If bacteria easily acquire resistance to the CPPs, the compounds may not be viable therapeutics, or would require a new approach with additional compound(s) for synergy and reduced risk of resistance emergence.
  + **Hypo**: A high barrier exists for the emergence of CPP resistant mutants
  + **Protocol**: Done by Purdue
    - Passage target microbes in the presence of increasing concentrations of drug over at least 15 passages. Determine MIC of resulting resistant strains. Sequence strains to determine genetic mechanism of resistance.
  + **Resources needed**: CPPs, microbes, culture reagents and supplies
  + **Estimated deadline: September 5 grant submission**

* **PK and Exploratory Tox**
  + Priority and Rationale: Lower priority for older CPP compounds. Higher for the GAP series. Determining the drug levels in tissue and serum following various dosing routes and exploratory tox following IV dosing will inform target indication and lead selection.
  + **Hypothesis**: GAP peptides are viable as systemic treatments.
  + **Protocol**: Done at TSRL. Dose mice IV/PO/SC with GAP peptide. Sample blood across multiple time points for plasma levels via LC-MSMS. Monitor for histamine response immediately after dosing. Harvest organs and blood after final time point for clinical and histopathologic analysis.
  + **Resources needed**: GAP peptide. Animals. Blood cards (if compatible with CPP). Funds for clinical labs as well as organ harvest and histology.
  + **Estimated deadline: September 5 grant submission**
* **Efficacy testing**
  + **Priority and Rationale:** High priority. Based on the potency results, the *in vivo* efficacy of the lead CPP candidates against extra- and intracellular pathogens, especially ESKAPE pathogens, will be determined. Demonstration of *in vivo* efficacy will advance CPP towards an IND application and further define
  + **Hypo**: CPPs are effective against a broad spectrum of G+ and G- bacteria *in vivo*, including intracellular pathogens
  + **Protocol**: Testing done at Purdue using wound infection model
  + **Resources needed**: CPPs will be synthesized and tested at Purdue
  + **Estimated deadline**: **September** 2019

# 928 – MetRS

* **Bring knowledge of field up to date**
  + **Estimated deadline**: 7/1/19
  + Maintain the Zotero Reference library continuously (ie add recently published and relevant papers as they are seen) for the project accordingly
* **Write code to allow for visual lead selection based on multiple parameters, including hERG data**
  + **Priority and Rationale:** High once hERG data available. We currently have 10 compounds under evaluation. We will need to narrow this down to move forward with a potential product. The code will also be adaptable to other projects.
  + **Hypothesis**: Visualizing the critical data for the MetRS compounds will reveal a lead candidate.
  + **Protocol**: Write annotated R code that builds a multi-parameter graph to compare pre-lead compounds based on accumulated data.
  + **Resources** **needed**: hERG data, solubility, Cmax, and potency data, time for coding
  + **Estimated** **deadline**: **5/1/19**
* **Solubility**
  + **Priority and Rationale:** High. Compound solubility data is necessary for lead determination and may inform PK observations.
  + **Hypothesis:** The MetRS compounds are acceptably soluble at pH 6.5 (Lowest pH for IV Bag)
  + **Protocol:** Incubate known amounds of compound in buffer at 37C for 24 hours. Measure amount in solution using LC-MSMS.
  + **Resources needed**: Compounds. Buffer. HPLC reagents and consumables. Waterbath.
  + **Estimated deadline: 3/8/19**

# 930 – Tri/TOB

* **Bring knowledge of field up to date**
  + **Estimated deadline**: (8/1/19)
  + Maintain the Zotero Reference library continuously (ie add recently published and relevant papers as they are seen) for the project accordingly
* **Resubmit the DFU grant, if needed (9/5/19)**
* **Solubility**
  + **Priority and Rationale:** High. Tri is not soluable in water, while tobramycin is very soluable. Solubility of the two compounds in various buffers will be determined to aid in determining a lead formulation.
  + **Hypothesis:** Tri and Tob will be compatible in a mixed formulation.
  + **Protocol:** Done at TSRL. Incubate known amounds of compound in lqiud formulations at 37C for 24 hours. Measure amount in solution using LC-MSMS.
  + **Resources needed**: Compounds. Buffer. HPLC methods, reagents, and consumables. Waterbath.
  + **Estimated deadline:** 9/31/19
* **PK**
  + **Priority and Rationale**: High, especially once a possible formulation available. The PK characteristics of inhaled and IV TOB/TRI needs to be determined. Ideally we will see very little systemic Tob or Tri following inhaled administration while IV dosing will provide characteristics of the combination in plasma. Lung lavages at necrospy could be used to determine if tissue levels are above the MIC.
  + **Hypothesis**: Inhaled Tob/Tri will not result in high levels in plasma.
  + **Protocol**: Done at TSRL. Mice or rats will be dosed intratracheally (IT) and IV with Tob/Tri. Serial blood sampling will be used to track drug levels in plasma dried blood spots if compatible.
  + **Resources needed**: Animals, IT administration training, Blood cards, Tob/Tri formulation, LC-MSMS methods, reagents, and consumables
  + **Estimated deadline**: 12/31/19
* **Efficacy**
  + **Priority and Rationale**: High. Demonstrating efficacy of inhaled Tob/Tri for respiratory infections is crucial to this project.
  + **Hypothesis**: Tob/Tri effectively clears infection in the mouse model of chronic pneumonia.
  + **Protocol**: Done at MSU. Mice are chronically infected with biofilms on agar beads. Once chronic infection established, mice are dosed via inhalation.
  + **Resources needed**: Chronically infected animals
  + **Estimated deadline**: 3/31/20
* **Tox**
  + **Priority and Rationale**: Low. We plan to rely largely on published data for both Tob and Tri safety. However, we will need demonstrate the safety of the combined product following inhalation.
  + **Hypothesis**: Inhaled Tob/Tri will not result in adverse events compared to the vehicle control.
  + **Protocol:** Done at TSRL. Animals would be dosed via inhalation (IT or nebulized) based on PK and Efficacy data. Tissues and blood will be harvested for clinical and histopathological analysis.
  + **Resources needed**: Tri/Tob, animals, necropsy and histopathology services, blood chemistry services.
  + **Estimated deadline:** 12/31/20

# 931 – Prodrugs of Foscarnet

* **Bring knowledge of field up to date**
  + **Estimated deadline**: 9/1/19
  + Maintain the Zotero Reference library continuously (ie add recently published and relevant papers as they are seen) for the project accordingly
* **Investigate other viral indications for Foscarnet** (12/31/19)
  + Requires
    - PK and biodistribution of Foscarnet-prodrug to confirm viability of indications
    - *In vitro* potency
* **Evaluate Foscarnet prodrugs as adjuvant to Fosfomycin antibacterial therapy (10/1/19**)
  + Ref: <https://www.ncbi.nlm.nih.gov/pubmed/28993329> - “additional toxicology studies are required to fully assess the feasibility of fosfomycin-PPF combinations, including proof-of-concept studies in an appropriate animal model”

# 932- Prodrugs of Sofosbuvir

* **Bring knowledge of field up to date**
  + **Estimated deadline**: 4/1/19
* **Screen Sofosbuvir for potency against non-Hepatitis RNA viruses**
  + **Priority and Rationale**: If sofosbuvir is potent against a broad range of viruses, we could modify the prodrug moiety to change the distribution of active drug in the body to effectively fight RNA virus infections in tissues beyond the liver.
  + **Hypo**: Sofosbuvir is effective against a broad spectrum of RNA viruses
  + **Protocol**: Done at U of F
  + **Resources needed**: SFB prodrugs sent to Ashley for screening, consumables for culturing, reagents for viral load assessment, precise cost not known at this time
  + **Estimated deadline**: **September grant submission**
* **Intracellular Conversion of SFB prodrugs**
  + **Priority and Rationale**: Ashley Brown reports different levels of SFB conversion to active form depending on cell type. If we want to alter SFB to act as a broad spectrum anti-viral, we will need to determine which target tissues can form active drug to determine our viral targets.
  + **Hypothesis**: Conversion of nucleoside prodrugs differs by tissue/cell type
  + **Protocol**: Treat cultured cells from target tissues with compound. Lyse cells and measure prodrug and metabolites per 106 cells.
  + **Resources needed**: Bioanalytical methods for prodrug and active drug (+metabolites), SFB prodrugs, cells, cell culture consumables
  + **Estimated deadline: September grant submission**
* **PK Studies**
  + **Priority and Rationale**: High. If our sofosbuvir prodrug can reach tissues other than the liver, it may be a viable product for viral indications beyond HepC
  + **Hypothesis**: Our prodrug moiety results in distribution to tissues beyond the liver at effective concentrations.
  + **Protocol**: Single-dose PK study. Mice will be dosed with SFB prodrug PO/IV. Blood will be samples serially using dried blood cards. Tissues will be collected at the study’s end to determine the levels of prodrug and SFB.
  + **Resources** **needed**: Animals. SFB prodrugs, blood cars, LC-MSMS methods, reagents and consumables.
  + **Estimated** **deadline**: 4**/1/19**

# Multi-Project Gaps

* **Determine feasibility and develop method for nucleoside or related prodrugs and their metabolites using column separation and alkaline phosphatase**
  + **Estimated** **deadline**:  **3/15/19**
* **Determine resource needs and re-establish in-house viral potency testing at TSRL** 
  + **Estimated** **deadline**:  **(8/1/19)**
  + **Protocol**: May be able to use Viral ToxGlo™ Assay (Promega, ~$500/100 mL) with our plate ready. More research needed.
* **Write an SOP for the use of Zotero to store and reference outside sources in reports, protocols, and grants.**
  + **Estimated** **deadline**:  **4/1/19**
* **Bring knowledge of field up to date for each project:**
  + **Priority and Rationale: higher priority**. Keeping up with the field will alert us to potential competitors and collaborators and changes in the commercial prospects of our projects. Will also make grant writing more efficient by having up to date and easily referenced knowledge ready for inclusion in grants and other relevant documents. Literature reviews could also be published in peer-reviewed journals to promote TSRL.
  + **Protocol:** Continuously monitor scientific publications for new papers relevant to our work. Summarize these papers once annually as a literature review.
  + **Resources needed**: Journal access and time to read and write
  + **Estimated deadline**: Literature review focused on one project or indication every 4-6 weeks

Template

* Exp
  + Priority and Rationale:
  + Hypothesis:
  + Protocol:
  + Resources needed:
  + Estimated deadline: