



SARS Therapeutics and Vaccine Approaches

*The role of Contract Research Organizations in
Vaccine Discovery and Development*

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The SARS Challenge At Southern Research Institute

- Understand the basic biology of SARS coronavirus
 - Identify drug targets
 - *in vitro* screening of antivirals
- Develop animal models of SARS coronavirus infection
 - Identify correlates of disease
 - pathogenesis
 - transmission
 - Identify correlates of immunity
 - Innate, cellular, and humoral responses
- Develop and evaluate potential antiviral compounds
- Develop and evaluate vaccine candidates



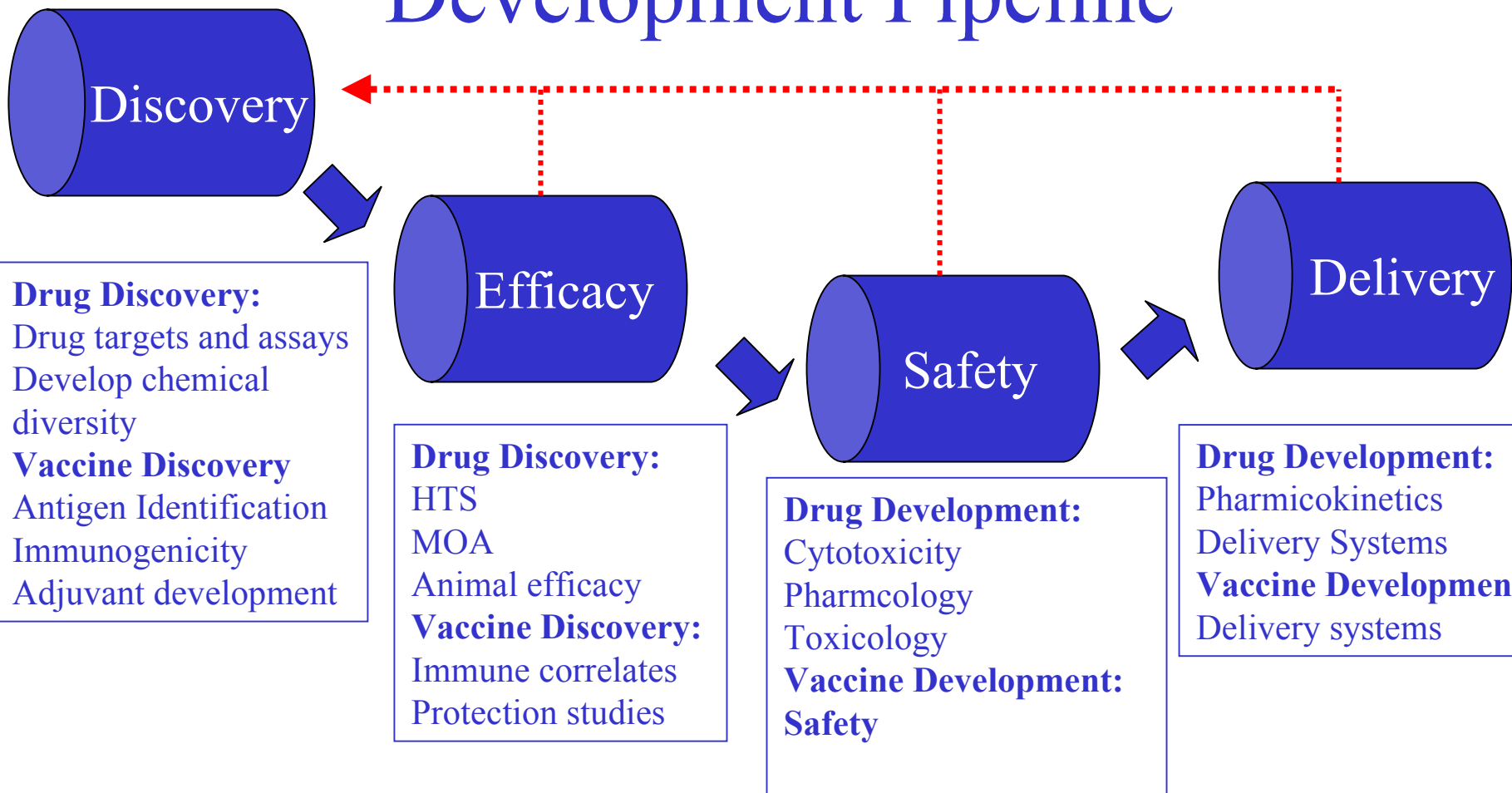
Strategy for Drug and Vaccine Discovery

Get to patients

...FAST...



Drug and Vaccine Discovery and Development Pipeline





SOUTHERN RESEARCH
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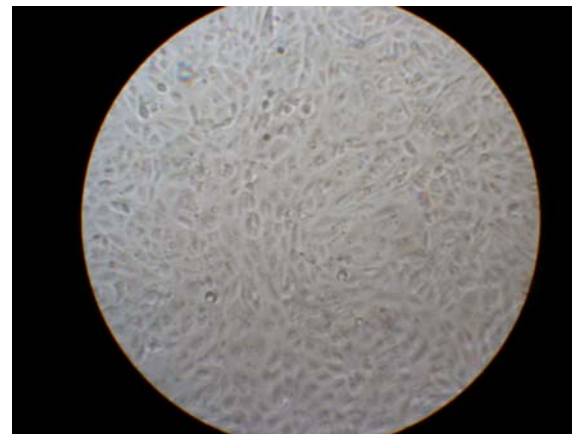


SARS Antiviral Discovery at Southern Research



Antiviral Screening Program

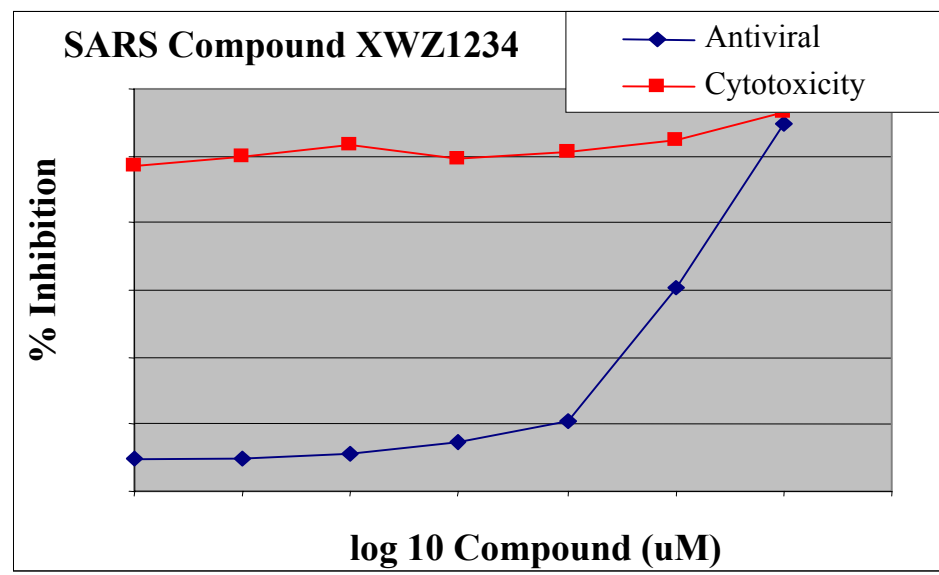
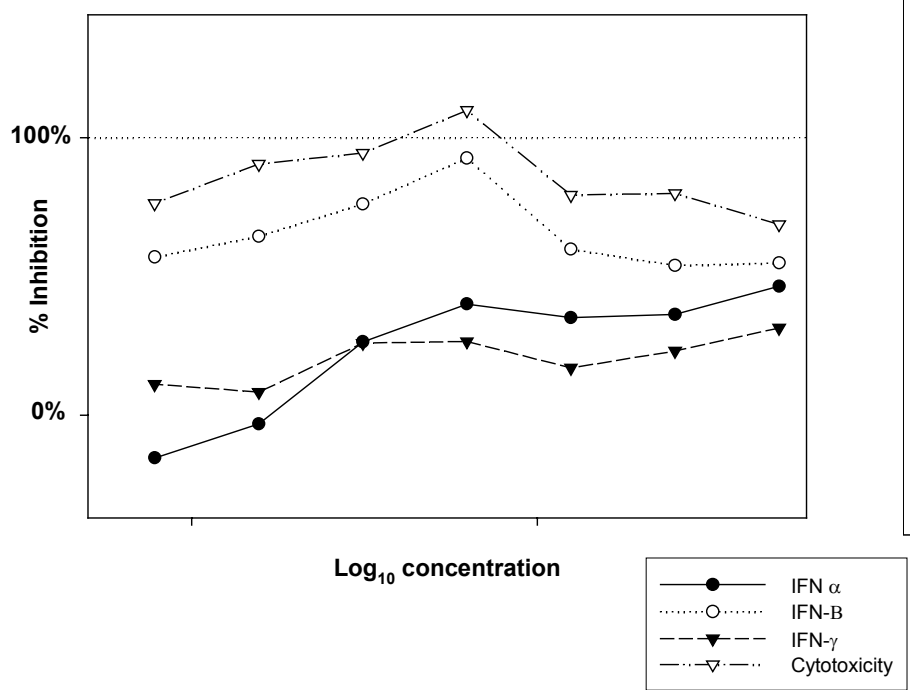
- *In vitro* system developed based on cytoprotection assay
- Almost 2500 compounds screened to date
- NIAID contract awarded (HepB/C/SARS Screening Program Award)
 - HTS program -160,000 per year to be screened
 - MOA assay development for leads generated from HTS
 - polymerase, protease, fusion, others





Early Results from Screening

Type I interferons vs. Toronto-2 virus
72 hour *in vitro* assay





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SARS Coronavirus Animal Model Development



Animal Model Development: What we know and what we still need to know

- Early studies with rodents were not successful in replicating SARS infection and/or pathology
- Non-human primates showed great value as a model of SARS infection and pathology
- The need for animal models is multi-faceted
 - SARS coronavirus pathology and natural history
 - Correlates of disease
 - Correlates of immunity
 - Virus-host interactions and pathology
 - Mechanisms of transmission
 - Antiviral efficacy
 - Vaccine efficacy



Model Development Status at Southern

- NHP model replicated and used for antiviral and pathology studies
 - Rhesus vs. cyno's
- Small animal studies are in progress to evaluate
 - mice
 - cotton rats - infection at early time points, pathology pending
 - ferrets-results similar to Ab with intranasal inoculation and transmission shown without direct contact of animals
 - small NHP in progress



Validation of a non-human primate model of SARS coronavirus infection



Experimental Design

- Two species of macaque
 - 4 rhesus macaques
 - 4 cynomolgous macaques
- Two routes of infection
 - Intratracheal (IT)
 - Intravenous (IV)
- Challenge with 1×10^7 PFU TOR2 SARS-CoV
- Monitor clinical signs
- Collect blood and swabs for clinical chemistry, virus and immunology studies
- Necropsy and collect tissues for virus load (Pfu/RT-PCR) and histopathology



Overall Results

- General observations:
 - I.T. infected animals showed respiratory signs early
 - Minor lethargy days 1-3 post-infection
 - No rash noted
- Body Weight:
 - normal weight gain
- Temperatures:
 - No significant changes except in IT animals
- Hematology:
 - One I.T. cyno showed increased WBC on Day 3 (6.5→31.7)
 - Transient drop in platelets (Day 3) in all but one animal
- Clinical Chemistry:
 - No changes



Virology

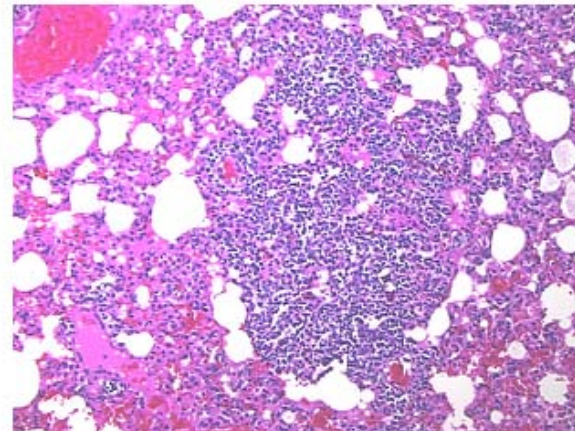
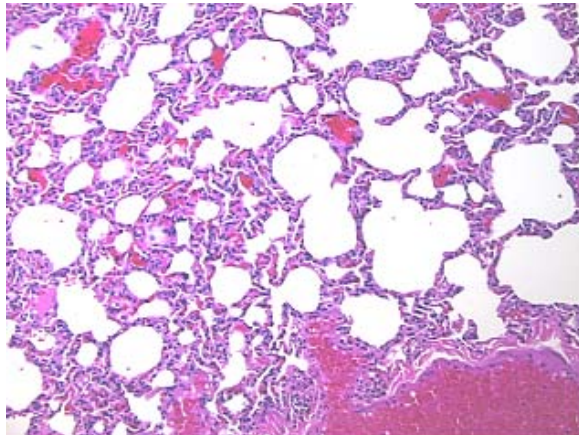
ID/Species	Route	Tissues					Nasal Swabs					Pharyngeal Swabs				
		Li	Lu	LN	Sp	Tr	0	3	5	7	12	0	3	5	7	12
17087(cy)	IT	ND	8	130	1	1	0.7	0.3	ND	8.4	NS	ND	ND	NS	ND	NS
17104 (cy)	IT	0.2	ND	2	ND	2	0	4.6	ND	ND	NS	ND	2.9	5.4	1.3	20
17103 (cy)	IV	22	1.4	2.5	110	ND	ND	NS	ND	ND	NS	ND	ND	ND	ND	NS
17179X (cy)	IV	ND	8.9	110	170	ND	ND	0.3	10	4.8	ND	0.2	0.3	1.5	0.3	NS
RQ4364 (rh)	IT	ND	1.2	7120	0.6	42	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
RQ4369 (rh)	IT	ND	ND	0.6	ND	ND	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
RQ4348 (rh)	IV	ND	ND	ND	ND	ND	0.7	0.6	1.3	0.5	NS	0.3	0.5	0.5	0.3	NS
RQ4354 (rh)	IV	ND	0.8	2	62	ND	NS	NS	NS	NS	NS	0.2	0.4	0.3	1	NS

1. NS: no sample received yet, ND: not detected, IP: in the process of analysis, "--": Not tested.
2. For tissue samples, Total RNAs were extracted from tissues after grounded in Trizol without addition of any carrier tRNA. 1 ug each total RNA was used to set up a 100 ul RT reaction (10 ng/ul). 10 ul out of 100 ul RT reaction was used for TaqMan analysis of the NP gene. The data represent the titers of NP sequence in 100 ng of total RNA.
3. For swab samples, Total RNA for each sample was co-ppted with either tRNA linear acrylamide (LA) and all used for RT reaction (100 ul reaction). 10 ul each of RT reactions was used for TaqMan



Summary

- Virus could only be detected by PCR analysis of RNA from tissues and/or swabs
- Virus cultured from lungs and nasal swabs
- CBC and clinical chemistry data were largely unremarkable
- There were no overt clinical symptoms of disease, severe lethargy, or failure to thrive among any of the animals
- The macaques infected via intratracheal route *did* present with occasional sneezing and coughing (Days 5-12), and one did show signs of pathology based on histopathologic examination



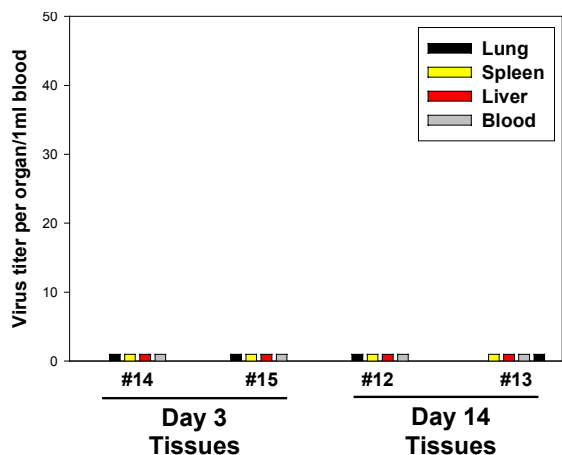


Approaches to address the question of murine susceptibility to SARS coronavirus infection

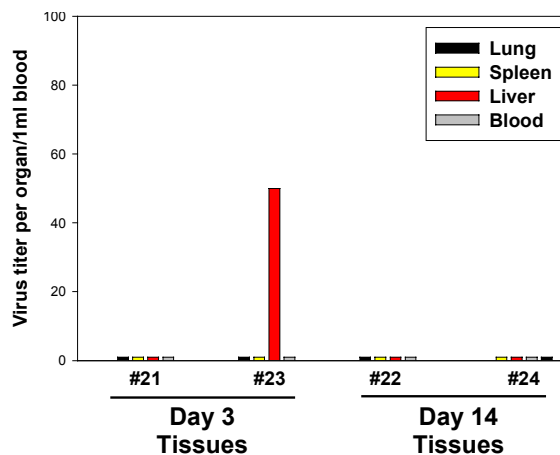
- Normal mice
- Immunocompetent inbred mice and *in vivo* neutralizing antibodies against IFN α/β
- IFN α/β receptor knockout mice
- **Other knockout mice**
 - Virus titer in tissues
 - RT-PCR in tissues (with UPENN)



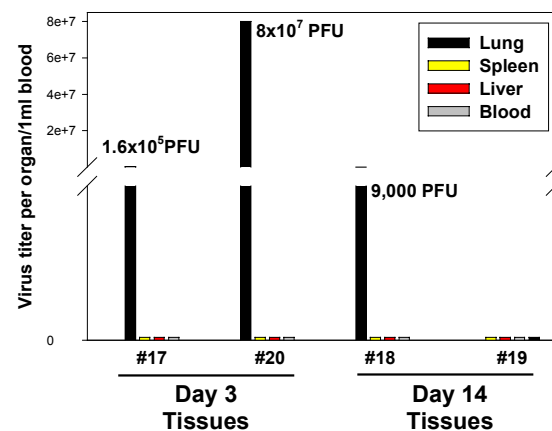
Knockout mouse tissue virus titers after SARS coronavirus infection



Ocular



Intraperitoneal



Intranasal



Detection of SARS virus in tissues at Day 14 post-infection by RT-PCR

Quantitative RT-PCR analysis of tissues from mice infected with SARS

Animal ID	Tissues analyzed		
	<u>Liver</u>	<u>Lung</u>	<u>Spleen</u>
#12 Ocular	ND	ND	ND
#13 Ocular	ND	ND	ND
#18 Intranasal	590	1,300,000	81
#19 Intranasal	56	7.4	1.1
#22 Intraperitoneal	63	ND	630
#24 Intraperitoneal	6.3	320	ND

- ND = Not detected
- 1 ug RNA each was used for RT and 1/10 of RT reaction (equivalent to 100 ng RNA) was used for PCR.



“Amateurs talk about strategy,
experts talk about logistics”

General Omar Bradley



Possible Vaccine Approaches

- Live attenuated viruses
 - infectious clone
 - serial passage virus
- Whole killed virus
- Subunit vaccines
 - S protein
 - N protein
 - M protein
 - Combinations of S/N/M
- Vectors
 - Adenovirus
 - Measles virus
 - bacterial vectors
- Genomic vaccines
- Other approaches



What vaccines are we working on or planning to evaluate?

- Live attenuated viruses
 - infectious clones
 - serial passage virus
- Subunit vaccines
 - S/N/M protein
 - plant virus-based expression
 - mammalian expression
 - bacterial expression
- Novel adjuvants
 - GPI 100
- Vectors
 - measles virus
 - adenovirus
 - alphavirus
- Genomic vaccines



Keys to successful product development-Vaccines

- Animal models
 - understanding of the natural history of SARS *in vivo*
- Ability to identify antigens or epitopes
 - *in vitro* and *in vivo* systems
- Platform technology to produce vaccine efficiently
- Reliable immune correlates
 - innate immunity
 - humoral immunity
 - cell-mediated immunity
- Animal models
 - understanding of the natural history of SARS *in vivo*
 - rodents for “screening”
 - NHP for final efficacy



Summary and Conclusions

- Correlation of human SARS data and NHP data is critical to define the immune and pathogenic correlates of infection and immunity to SARS coronavirus
- A range of approaches to SARS vaccine development are necessary to allow for the use of novel technology platforms for SARS vaccines
- Animal models are critical to advancing therapeutics and vaccines for SARS
- BSL-3 and ABSL-3 space and expertise is not sufficient to support robust programs that require NHP challenge
- Live vaccines or whole killed will require significant GMP BSL-3 space for vaccine production



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