Available online at www.sciencedirect.com
ScienceDirect
Antiviral Research 78 (2008) 51–59
Antiviral Research
www.elsevier.com/locate/antiviral

NIAID resources for developing new therapies for severe viral infections

Heather Greenstone, Beth Spinelli, Christopher Tseng, Susan Peacock, Katherine Taylor, Catherine Laughlin *

Division of Microbiology and Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, United States

Received 10 October 2007; accepted 12 October 2007

Abstract

Severe viral infections, including hemorrhagic fever and encephalitis, occur throughout the world, but are most prevalent in developing areas that are most vulnerable to infectious diseases. Some of these can also infect related species as illustrated by the threatened extinction of gorillas by Ebola infection in west and central Africa. There are no safe and effective treatments available for these serious infections. In addition to the logistical difficulties inherent in developing a drug for infections that are sporadic and occur mainly in the third world, there is the overwhelming barrier of no hope for return on investment to encourage the pharmaceutical industry to address these unmet medical needs. Therefore, the National Institute of Allergy and infectious Disease (NIAID) has developed and supported a variety of programs and resources to provide assistance and lower the barrier for those who undertake these difficult challenges. The primary programs relevant to the development of therapies for severe viral infections are described and three case studies illustrate how they have been used. In addition, contact information for accessing these resources is supplied.
Published by Elsevier B.V.

Keywords: Adenoviruses; Anti-inflammatory; Antiviral testing program; Avian H5N1; BEIR; Biodefense and emerging infections repository; BK virus; BLA; BSL-4 containment; Cationic lipid–DNA complexes (CLDC); Chemical synthesis; Clinical evaluations; Cloned genes; Cowpox virus; Cytomegalovirus; Dengue; Determination of exceptional circumstances (DEC); Epstein-Barr virus; Filoviruses; Formulation; Hamster-scrapie; Hepatitis B virus; Hepatitis C virus; Herpes simplex type 1 and type 2; Highly pathogenic RNA viruses; HIV; Human herpes virus 6; Human herpes virus 8; Immunomodulatory; *In vivo* testing submission form; IND; Influenza type A; Intellectual property option (IP option); Intellectual property; Junin; Juvaris BioTherapeutics; Lassa fever; Manufacturing; Measles; Medicinal chemistry; Monoclonal antibodies; NDA; NIAID Category A, B and C; NIAID; Nucleic acids; Orthopoxviruses; Papillomaviruses; Parainfluenza; Patent protection; Pharmacodynamic; Pharmacokinetic; Phase I clinical trials; Pichinde; Radiolabeled compounds; Recombinant proteins; Respiratory syncytial virus; Rhinoviruses; Rift valley fever; Screening agreement; Services for the preclinical development of therapeutic agents; Severe acute respiratory syndrome (SARS)-associated coronavirus; Synthesis/resynthesis; Synthetic peptides; Tacaribe; Vaccinia virus; Varicella-Zoster virus; Venezuelan equine encephalitis; West nile; Western equine encephalitis viruses; Yellow fever

1. Introduction

There are many reasons why an arsenal of therapies for severe viral infections, including those caused by highly pathogenic RNA viruses, does not exist. The primary reason applies to the development of safe and effective treatments for any infections caused by viruses. Since viruses can be described as obligate intracellular parasites that subvert cellular metabolic and reproductive processes for their own persistence and replication, the difficulty in identifying a drug target that would derail viral replication without causing concurrent host toxicity is easily appreciated. In fact the idea that the identification of a safe and effective antiviral drug was by definition impossible, was scientific dogma for many years. This mistaken ideology was first brought into question by the discovery and development of amantadine for influenza and acyclovir as a treatment for herpes simplex. It was finally laid to rest with the emergence of HIV and the AIDS epidemic, and the astonishingly rapid accomplishment of the development of 25 drugs against five separate HIV viral targets. However the question remains as to why similar successes have not been enjoyed for the other RNA viruses that cause highly pathogenic infections.

Unfortunately, many of the reasons are as clear as they are difficult to solve. First, many of these viruses are endemic or emerge

* Corresponding author: Tel.: +1 301 496 7453; fax: +1 301 480 1594.
E-mail address: claughlin@niaid.nih.gov (C. Laughlin).

0166-3542/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.antiviral.2007.10.006

sporadically in parts of the world with inadequate public health resources. These circumstances usually correlate with a lack of economic incentive for the pharmaceutical industry. Secondly, many of the diseases they cause are rare which complicates the clinical evaluation of potential therapies. Most importantly, many of these infections cause high morbidity and/or mortality and therefore research on them requires access to high-level biological containment. Finally, the host response to infection may make a significant contribution to the pathology such that an antiviral monotherapy may not be sufficient for disease control.

Since these infections have a serious global impact as naturally emerging infections and also have the potential for misuse by bioterrorists, the U.S. government, and others have developed publicly available resources to facilitate the discovery and development of therapies for these infections. This article will describe many of these supported by the National Institute for Allergy and Infectious Diseases (NIAID) that run the gamut from chemical synthesis to limited clinical evaluations. Contact information for the various programs is provided and intellectual property issues are discussed. The article ends with several examples of how specific resources were accessed by drug developers and provided data to fill gaps in the development plans of these therapies.

2. Preclinical programs: *in vitro* screening, animal model evaluation, and BEI repository

The Antiviral Testing Program of the NIAID supports *in vitro* screening systems and animal models of human viral infections for the assessment of the efficacy and toxicity of potential therapeutics for important human viral infections. The main objective of this program is to facilitate the identification of antiviral agents with the potential for treatment of viral infections of public health importance, including those for newly emerging infections, and those that are a low priority for the pharmaceutical industry. The services are free for suppliers interested in submitting compounds to be evaluated for antiviral activity. In general, compounds are put through *in vitro* evaluations prior to the consideration of evaluation in relevant animal models. NIAID encourages academic and industrial investigators to take advantage of this program. Since the primary purpose of the program is to encourage successful product development, NIAID ensures that the compound supplier's intellectual property rights are protected. A properly executed Screening Agreement is required for both *in vitro* and *in vivo* evaluations under this program. The primary purpose of this agreement is to assure both that NIAID's use of the supplied compound is clearly defined and to protect the rights of the supplier. NIAID has developed a template agreement that can be modified with the assent of lawyers for both NIAID and the supplier.

2.1. In vitro testing

The *in vitro* screening program, initiated in 1989, is designed to examine pure compounds, but on rare occasions, will also examine biological extracts if there is a compelling rationale. Due to limited resources, the NIAID does not support the fractionation of extracts, and the supplier must demonstrate the capability for such fractionation before extracts will be accepted into the program. Although exceptions are occasionally made, the chemical identity or characterization of compounds to be screened must be released to the NIAID staff manager who will keep this information confidential. This is necessary so that the same compounds are not screened repeatedly. All of the assays are replication based. The assays for influenza and SARS are also available in a high throughput format. The viruses available for screening under this program are:

- Biodefense RNA viruses: Venezuelan equine encephalitis, Punta Toro, Rift Valley fever, Pichinde, Junin, Tacaribe, dengue, yellow fever, West Nile.
- Orthopoxviruses: Vaccinia virus, cowpox virus.
- Herpesviruses: Herpes simplex virus types 1 and 2, Varicella-Zoster virus, cytomegalovirus, Epstein-Barr virus, human herpes virus 6, and human herpes virus 8.
- Respiratory viruses: Influenza type A (including avian H5N1) and type B, parainfluenza, respiratory syncytial virus, measles, rhinoviruses, adenoviruses, and severe acute respiratory syndrome (SARS)-associated coronavirus.
- Hepatitis B virus.
- Hepatitis C virus.
- Papillomaviruses.
- BK virus.

The compound sponsor generally receives the data back within 2 months. The information provided to the sponsor on the data sheet includes the median effective concentration (EC50) and the concentration that reduces cell viability by 50% (CC50). A positive and a negative control are always run in parallel. More information and submission information can be found at: http://niaid-aacf.org/.

2.2. In vivo testing

NIAID also offers an antiviral evaluation service using a number of small animal models of human viral disease. The models currently include mouse and/or hamster models of biodefense-related viruses such as Punta Toro, Pichinde, yellow fever, West Nile, influenza A (including avian H5N1), and Venezuelan and Western equine encephalitis viruses. Some of the biodefense-related viral models serve as surrogates for their more hazardous counterparts such as Punta Toro as a surrogate for Rift Valley fever and Pichinde as a surrogate for Lassa fever. The program also includes less exotic pathogens such as mouse models of herpesviruses, mouse or rabbit models of papillomavirus, cotton rat models of paramyxoviruses, and mouse models of orthopoxviruses. In addition, the program offers a transgenic mouse model of hamster-scrapie (see detailed descriptions of the models on the website referenced below). The NIAID contract laboratories are staffed by established investigators who possess a strong antiviral research background and publication record in their respective animal models. Often there are several different models available for an individual pathogen and these may vary in method of virus inoculation or animal mortality.

It is also often possible to evaluate different methods of drug delivery when appropriate such as intraperitoneal, topical, oral or intravenous as well as a range of doses and regimens. The first experiment in a series is usually designed to give the drug every advantage to show efficacy so the candidate therapy may be administered at the same time as the virus is inoculated or even before. Endpoints can include clinical parameters such as mortality, weight and oxygen saturation, and laboratory values such as virus titers in various organs. Therapeutic combinations can also be evaluated in these models. Table 1 lists a selection of currently available models. Antiviral evaluations for filoviruses are not currently offered but may be done in collaboration with the Department of Defense or some academic investigators.

To gain access to the testing service, submitters must complete an "*In Vivo* Testing Submission Form" which provides the NIAID with background information on the product being submitted. The information is used by NIAID staff to select and prioritize materials for testing. Criteria for prioritization include addressing an unmet medical need, having novel chemical or desirable pharmacokinetic characteristics, utilizing a novel therapeutic strategy and having pharmaceutical backing for further development. Often, the materials submitted to the *in vivo* testing program are those that were "hits" in the *in vitro* screening process. Exceptions to this include products that are anti-inflammatory or immunomodulatory in nature. Such products would not be expected to manifest antiviral activity *in vitro*, so these products are considered by the NIAID for direct submission to the animal model program. After a product has been selected for testing, the compound sponsor, the NIAID contractor, and NIAID program staff work together to develop specific protocols to evaluate the product. The experiments are conducted in a stepwise fashion with continual involvement of the three parties. Rarely, a sponsor does not want to be involved in planning for each specific study. In those cases, NIAID staff and the animal model contractor will develop the plan and then discuss the results with the sponsor.

Although the capability to chemically assay animal-derived specimens for the presence of the product and/or metabolites is not covered under these contracts, the animal model contractors can harvest tissue/blood samples at appropriate times so that the compound sponsor can arrange to have assays done elsewhere to collect pharmacokinetic and pharmacodynamic data. The NIAID strongly supports the publication of significant findings made under the auspices of the NIAID antiviral testing program although publication may be delayed if the patent protection process is still in progress. More information can be found at: http://niaid-aacf.org/animals.htm and in Table 1.

Table 1
NIAID supported animal models of human viral infections

Virus	Species
West Nile	Mouse/hamster (Morrey et al., 2004a,b, 2006, 2007)
Punta Toro	Mouse/hamster (Gowen et al., 2006a)
Pichinde	Hamster (Gowen et al., 2006b)
Yellow fever	Hamster (Julander et al., 2007a,b)
Venezuelan equine encephalitis	Mouse
Western equine encephalitis	Mouse/hamster (Julander et al., 2007c)
Herpes Simplex-1	Mouse/rat/newborn guinea pig (Prichard et al., 2006; Quenelle et al., 2006a)
Herpes Simplex-2	Mouse/guinea pig (Bernstein and Harrison, 1989; Bernstein et al., 2001, 2003; Bourne et al., 1999; Milligan and Bernstein, 1997; Quenelle et al., 2006a; Stanberry et al., 1990)
Murine cytomegalovirus	Mouse (Kern et al., 2004b)
human cytomegalovirus	SCID/SCID-hu-Ret/SCID-hu-thy/liv (Bravo et al., 2007; Kern et al., 2004a)
Guinea pig cytomegalovirus	Normal, immunocompromised, newborn, or pregnant guinea pig (Bourne et al., 2000; Bravo et al., 2003, 2006)
Vaccinia	Mouse (Quenelle et al., 2004a,b, 2006b; Smee et al., 2007a,b, 2004b)
Cowpox	Mouse (Quenelle et al., 2007, 2003, 2004a,b, 2006b)
Ectromelia	Mouse (Buller et al., 2004; Parker et al., 2008; Schriewer et al., 2004)
Monkeypox	Mouse (Stat1−/−)/African Dormouse
Influenza A (H1N1, H3N2, H5N1 (not Asian high path))	Mouse (Sidwell et al., 2007; Smee et al., 2006)
Influenza B	Mouse (Smee et al., 2004a)
SARS-CoV	Mouse (Barnard et al., 2006a; Barnard et al., 2006b)
SARS-CoV	Hamster
Respiratory syncytial virus	Cotton rats (Wyde et al., 2003, 2005b)
Measles	Cotton rats (Wyde et al., 2000a)
Human metapneumovirus	Cotton rats (Wyde et al., 2005a)
Parainfluenza-3	Cotton rats (Wyde et al., 2000b)
Cottontail rabbit papillomavirus	New Zealand white rabbit (Christensen et al., 2001, 2000)
Rabbit oral papillomavirus	New Zealand white rabbit (Christensen, 2005)
Human papillomavirus 6, 11 (and as secondary testing) 16	Human HFF-SCID mouse (Bonnez, 2005; Iyer et al., 2002)
Hamster scrapie	Transgenic mouse (Kocisko et al., 2006)

NIAID has these models available, free of charge, to investigators whose products are accepted into the program. References are included to provide more information on the model and to provide examples of how these models have been used.

2.3. High containment evaluations

The NIAID is in the process of constructing two BSL-4 containment facilities in the continental United States and two more as part of already established government facilities. The capabilities of these laboratories will be accessible to qualified investigators and institutions although specific plans for managing access and determining priorities have not been finalized (as of November, 2007 when this article was prepared). Sponsors of drugs, whose development would require the use of these facilities, should contact an NIAID Program Officer (Table 2) about their availability.

2.4. Biodefense and emerging infections repository

Another DMID resource is the Biodefense and Emerging Infections Research Resources Repository (BEIR). This resource provides unique and quality-assured Biodefense and emerging infection reagents and resources to the scientific community. The mission of BEIR is to acquire, authenticate, produce/expand, preserve and distribute NIAID Categories A, B, and C research and reference reagents (up to BSL-3) to investigators for basic research and production of improved diagnostic tests, vaccines, and therapies. Some examples of the reagents available through BEIR are bacteria, protists, rickettsia, viruses, fungi, cell lines, toxins, recombinant proteins, synthetic peptides, monoclonal and polyclonal antibodies, SCM and nucleic acids (genomic material and cloned genes). These reagents are available free of charge to registered investigators. Acquisition of these reagents is one of the critically necessary and challenging tasks for BEIR. Therefore, investigators are highly encouraged to deposit reagents into BEIR to provide access/use of materials, relief from the burden of distributing reagents, and to provide secure storage of valuable reagents. In addition, BEIR has the capability of contracting for the preparation of specific reagents. If there is a reagent needed to advance a specific research area, contact an NIAID program officer or use the "contact" option at the BEIR homepage below.

To view the catalog please visit http://www.beiresources.org/. For other funding opportunities available through NIAID, please visit: http://www.niaid.nih.gov/ncn/budget/default.htm.

Table 2
DMID, NIAID virology and Drug Development Program Officers

Branch	Program name	Program Officer	E-mail
VB	Acute Viral Diseases	Cassetti, Cristina	ccassetti@mail.nih.gov
VB	Emerging Viral Diseases	Repik, Patricia	prepik@niaid.nih.gov
VB	Poxvirus and Other Acute Viruses	Challberg, Mark	mchallberg@mail.nih.gov
VB	International Clinical Research and Dengue Program	Cassetti, Cristina	ccassetti@mail.nih.gov
VB	Persistent Viral Diseases and ICIDR Research Program	Park, Eun-Chung	epark@niaid.nih.gov
VB	Herpesvirus and Prion Research	Beisel, Christopher	cbeisel@niaid.nih.gov
STI	Topical Microbicides Development and Evaluation	Deal, Carolyn	cdeal@mail.nih.gov
STI	Viral and Protozoan STDs (Program phased out)	Hiltke, Thomas	thiltke@niaid.nih.gov
RDB	Influenza, SARS, and Related Viral Respiratory Diseases Program	Levandowski, Roland	levandowskir@niaid.nih.gov
RDB	Influenza Vaccine Research Program	Cho, David	choda@niaid.nih.gov
RDB	Influenza Basic Research Program	Lacourciere, Karen	lacourcierek@niaid.nih.gov
RDB	Human Coronavirus Research Program	Cassels, Frederick	casselsf@niaid.nih.gov
RDB	Select Paramyxovirus Research Program	Kim, Sonnie	skim@niaid.nih.gov
RDB	RSV and Human Metapneumovirus Research	Cho, David	choda@niaid.nih.gov
RDB	Influenza Therapeutics Research Program	Krafft, Amy	kraffta@niaid.nih.gov
RDB	Influenza Diagnostics Research Program	Lacourciere, Karen	lacourcierek@niaid.nih.gov
VB	Antiviral Research, Analytical Chemistry and Clinical Trials Program	Tseng, Christopher	ctseng@mail.nih.gov
VB	Emerging RNA Antivirals and Chemistry Program	Tseng, Christopher	ctseng@mail.nih.gov
VB	Antivirals for Emerging DNA Viruses Program	Greenstone, Heather	hgreenstone@niaid.nih.gov
VB	Non-Emerging Antivirals and Clinical Trials Program	Dempsey, Walla	wdempsey@mail.nih.gov
VB	Vaccine Clinical Trials Program	Dempsey, Walla	wdempsey@mail.nih.gov
EHDB	Hepatic Diseases	Koshy, Rajen	rkoshy@niaid.nih.gov
EHDB	Viral Hepatitis A, C, and E	Koshy, Rajen	rkoshy@niaid.nih.gov
EHDB	Hepadnaviruses-HBV, Hepatitis Delta Virus	Berard, Diana	dberard@mail.nih.gov
RDB	Bacterial and Viral Respiratory Diseases; Maternal Immunization	Rubin, Fran	frubin@mail.nih.gov
EHDB	Enteric Diseases Program (Viruses)	Berard, Diana	dberard@mail.nih.gov
RDB	Vaccine Delivery Platform Technology Program	Khambaty, Farukh	khambatyf@niaid.nih.gov
OBRA	Biodefense Reagent Repository Program	Peacock, Susan	peacocksusan@niaid.nih.gov
OBRA	In Vitro and Animal Models for Biodefense and Emerging Diseases Program	Hewitt, Judith	jhewitt@niaid.nih.gov
OBRA	Biodefense Advanced Vaccines and Biologicals Development Program	Nuzum, Ed.	enuzum@mail.nih.gov
OBRA	Biodefense Advanced Drug Development Program	Taylor, Katherine	kataylor@mail.nih.gov
OBRA	Biodefense Advanced Drug Development Program	Spinelli, Beth	spinellb@mail.nih.gov

VB: Virology Branch; STI: Sexually Transmitted Infections Branch; RDB: Respiratory Diseases Branch; EHDB: Enteric and Hepatic Diseases Branch; OBRA: Office of Biodefense Research Affairs. Program Officers names, e-mail addresses and program titles are provided for staff who manage some aspect of antiviral drug development. Contact them to inquire about possible NIAID resources appropriate for antiviral drug development programs.

3. Preclinical services

Despite the success of the *in vitro* and animal model programs at identifying compounds with therapeutic potential, it was clear that many promising compounds do not achieve their developmental potential because many sponsors lacked the resources to support a full developmental path. Therefore, in 2006, NIAID established a comprehensive resource to provide an array of services to support preclinical development of therapeutic agents identified through NIAID funded grants and contracts, academia, the private sector, or other sources selected by the NIAID Program staff. This NIAID research contract known as the Services for the Preclinical Development of Therapeutic Agents offers a collection of preclinical services, such as synthesis/resynthesis, medicinal chemistry, formulation, manufacturing, and packaging, as well as *in vivo* safety, pharmacology and toxicology studies required to support clinical use in humans or IND/NDA/BLA filing. Working together with the compound sponsors, NIAID contract product developers and NIAID staff can also provide specific preclinical product development plans and evaluation of existing product development plans.

The services offered are intended to fill specific gaps in the overall development of a product. Examples of the types of services that have been provided for typical small molecule therapeutics include compound synthesis, formulation and manufacturing, dose range finding in rodents/non-rodents, rat PK and toxicology, dog PK and toxicology and absorption, distribution, metabolism, and excretion (ADME). There are, however, more sophisticated toxicology studies also available, such as genotoxicity, reproductive toxicology and other studies that may entail specialized capabilities. Investigators may benefit from the synthesis of their candidate drugs and drug analogs in quantities sufficient for subsequent biological and biochemical analyses, and this can include radiolabeled compounds. Additionally, investigators that have a structural lead series of compounds that have exhibited efficacy may use these contracts to pursue lead optimization and development services. These involve the generation of a lead optimization scheme for generating chemical analogues or the use of in silico systems to predict ADME.

The preclinical product development planning and evaluation service has been found to be extremely beneficial to investigators who are new to product development and who are unfamiliar with the regulatory requirements that need to be fulfilled to progress to an IND, NDA or BLA. An investigator with a lead compound and an understanding of the desired indication can also request the preparation of a specific developmental plan for a product. This process requires the investigator to present an overview of the studies that have been performed and to disclose the existing data. Using this information the contractor will develop an overall preclinical product development plan for the specified therapeutic. The report will include a brief description of the studies needed to begin Phase I clinical studies, a list of go no/go decision points, indications when interaction with the FDA should take place, and an estimated cost for each step. This service has proved useful to many programs regardless of the stage in the drug development process.

The specific services requested depend on the status of the individual candidate(s) as part of an overall product development plan and/or regulatory submission plan to the FDA. The administrative procedure for requesting a service is to first contact the NIAID Program Officer responsible for the specific programmatic area (a list is provided in Table 2). Investigators should work with their NIAID Program Officer to identify studies that will aid the drug program to reach the stage of a pre-IND meeting with the FDA, be more competitive for alternate funding, or to fulfill an FDA requirement for an NDA or BLA enabling study. All requests are considered on the basis of NIAID priorities, technical feasibility and the availability of funding.

4. Clinical research

Although researchers frequently are able to obtain sufficient funding for *in vitro*, animal efficacy studies and other preclinical testing of a new compound, support may not be available for more expensive clinical trials. NIAID is in the process of advertising for a Clinical Trials Unit to perform Phase I clinical trials for priority compounds whose sponsor cannot financially support these studies. If the budget permits, an award may be made in 2008. Contact an NIAID program officer for more information. An NIAID program person may also know of other options for acquiring resources for clinical testing. A current listing of NIAID Program Officers for virology programs is provided in Table 2.

5. Intellectual property and confidentiality

It has been NIAID's experience that some providers of materials may be hesitant to submit their proprietary products to a program where there is risk that government contractors may obtain rights to inventions relating to the provider's material. Therefore, NIAID has procedures in place to help assure Providers that their rights will not be compromised if they submit their Material for evaluation and development. Some of these provisions are incorporated into the contracts that NIAID holds with service providers, and others are in the form of separate agreements between NIAID and the contractors or NIAID and the investigators.

For all of its service contracts involving the use of third party proprietary material, NIAID either has in place or is in the process of pursuing a determination of exceptional circumstances (DEC). The DEC requires the contractor and its subcontractors to assign their rights under the Bayh–Dole Act in any subject inventions that use or incorporate submitted material to NIAID or to the provider as designated by NIAID, or otherwise dispose off or transfer those rights as directed by NIAID.

For some of the contracts in which a DEC is not yet in effect, an intellectual property (IP) option has been incorporated into the contract. The IP option offers the provider an option to negotiate an exclusive license to any NIAID contractor inventions using provider's material. This includes inventions either conceived or first actually reduced to practice by the contractor in the performance of work done under the contract using the provider's material.

Another concern amongst third party providers is confidentiality of data and information provided by the owner of the material. There are several procedures in place to protect confidentiality of proprietary information. In general, the standard NIAID contracts include a clause that states that all data and other information provided by a third party supplier or the NIAID project officer marked as confidential should be treated as confidential unless specifically identified as non-confidential in writing by the NIAID project officer. Last, NIAID would not prevent the submitter from directly negotiating separate terms and conditions with the contractors, as long as such terms do not conflict with the pre-negotiated contract that NIAID has in place with the contractors.

The intention of the various provisions described above is to protect the intellectual property and other proprietary rights of the providers. The DMID Program Officers are available to discuss which procedures are implemented for each contract and to address concerns.

6. Case studies

Although the 21st century has barely begun, its first few years have already witnessed the emergence and spread of West Nile virus in the United States, the sudden emergence of SARS-associated coronavirus in Asia, and the threat of a potential global influenza pandemic caused by a highly pathogenic avian influenza virus. Moreover, there is the unthinkable threat of bioterrorism faced by our society today. In response to these emerging public health issues, NIAID preclinical programs have been able to rapidly establish antiviral assays and animal models in response (Barnard et al., 2004; Morrey et al., 2002; Noah et al., 2007; Severson et al., 2007; Smee et al., 2002) and to adjust the use of resources to address new needs. Although only two of the resources described have existed for more than 6 years, and many of the models relevant to biodefense are new as well, a few examples of how these resources have been used may help the reader understand how the NIAID can assist companies attempting to develop new drugs against severe viral infections. An additional example of NIAID assistance with the development of a novel therapy for poxvirus infections has recently been described (Bolken and Hruby, 2007).

6.1. Case study 1

Juvaris BioTherapeutics, a small company, enrolled with the NIAID antiviral screening program in July, 2004. This company is developing immunotherapeutic and vaccine products for the treatment and prevention of human diseases. The company and collaborators have shown that cationic lipid–DNA complexes (CLDC) are able to activate the innate and adaptive immune responses, in particular the induction of therapeutic quantities of TH1 cytokines as well as humoral, and cell-mediated immunity following parenteral or mucosal administration. Initially, the breadth of data the company had developed using the CLDC technology was focused on the treatment of cancer in animal models and dogs with naturally occurring tumors (Dow et al., 1999; Kamstock et al., 2007; U'Ren et al., 2007).

Upon entry into the screening program, it was jointly decided among the company, senior scientists under the Utah State University animal models contract, and the program officer to test the protective effect of CLDC treatment in the Punta Toro virus infection murine model. Initial studies in the laboratory of Dr. Brian Gowen at Utah State demonstrated 100% protection against Punta Toro virus infection when CLDC were administered either prophylactically or therapeutically (Gowen et al., 2006a). The results of these studies and the resulting publication began a change in Juvaris corporate strategy as the management team began to focus more efforts on immunotherapeutic treatment applications for infectious diseases.

The combination of disease-specific antigens with CLDC produces vaccines capable of activating robust antibody and T-cell responses. In herpes simplex virus-2 (HSV-2) studies conducted under contract at Cincinnati Children's Hospital Medical Center, in the laboratory of Dr. David Bernstein and Dr. Rhonda Cardin, mice immunized with CLDC combined with an enriched HSV-2 glycoprotein lysate or recombinant gD2 protein have shown significant reduction in viral load and erythema as well as significantly increased survival (manuscript in preparation). Juvaris BioTherapeutics is currently evaluating a clinical development strategy for HSV-2 therapeutic and prophylactic vaccination as a result of the successful outcome of these studies involving the NIAID-sponsored program.

The company has used the data generated from the NIAID Antiviral Screening Program in support of multiple successful applications for financial backing from both governmental and non-governmental entities. In addition, the NIAID-Juvaris collaborative studies have generated substantial interest from major pharmaceutical groups.

6.2. Case study 2

MacroGenics, Inc. enlisted in the NIAID antiviral testing program in June 2005, with the submission of a humanized West Nile Virus-neutralizing antibody, hE16. Under the NIAID contract with Utah State University, Dr. John Morrey and colleagues discovered that hE16 could significantly protect hamsters from a lethal challenge with West Nile Virus even when the antibody was administered intraperitoneally 5 days post-viral injection, at a timepoint when the virus had already infected neurons in the brain (Morrey et al., 2006). However, there is a time-window that limits the therapeutic efficacy of hE16 in this model (Morrey et al., 2007). Further work under this collaboration established that WNV can undergo retrograde axonal transport, and that hE16 can prevent paralysis when administered 1 day after intra-sciatic nerve injection (Samuel et al., 2007).

The preclinical data obtained through the NIAID antiviral testing program was incorporated into a proposal the company submitted to a NIAID contract program which provides support to companies undergoing later stage product development. In September 2006, Macrogenics was awarded a 5-year contract under this program for further development of the monoclonal antibody including manufacturing through Phase 2 clinical trials. The preclinical data obtained through the NIAID antiviral testing program was included in an IND submitted to the FDA

in May 2007. In July 2007, the company announced that the IND for this antibody is active. A Phase 1 clinical trial of the humanized West Nile Virus-neutralizing monoclonal antibody began in August 2007.

6.3. Case study 3

The Toyama Chemical Company registered with the NIAID antiviral testing program in January, 2004. They submitted T-705, a pyrazine derivative that was reported initially by Furuta and co-workers at Toyama Chemical Co. as having activities against influenza viruses (Furuta et al., 2002; Takahashi et al., 2003). In collaboration with the company, the testing program further demonstrated its activities against the H5N1 viruses (Sidwell et al., 2007) and a panel of bunyaviruses and arenaviruses (Gowen et al., 2007).

After completion of a number of *in vitro* studies, *in vivo* testing of Toyama's lead compound, T-705, was performed in the mouse model for influenza. In the contract laboratory at Utah State University, the principle investigator at that time, Dr. Robert Sidwell and colleagues discovered for the first time that T-705 was highly active in mice lethally infected with influenza A/Duck/MN/1525/81 (H5N1) (Sidwell et al., 2007). When administered per os 1-h post-virus exposure, once, twice, or four times daily, dosages from 30 to 300 mg/kg/day were well tolerated; each prevented death, lessened decline of arterial oxygen saturation and inhibited lung consolidation and lung virus titers. The four times per day treatments at 300 mg/kg/day were also found to be efficacious when treatment was delayed 96 h after virus exposure. Remarkably, single T-705 treatments administered up to 60 h after virus exposure prevented deaths and arterial oxygen saturation decline. Altogether, over 10 experiments were conducted for the Toyama Chemical Company on T-705 versus influenza in the mouse model. The preclinical efficacy data from these experiments were used to support Toyama's IND submissions in both Japan and the United States. Safety evaluations in humans began in Japan in January 2007, and in the U.S. shortly thereafter. The NIAID is continuing to support the investigation of T-705 in combination with other antivirals and in combination with substances which may have an effect on immune modulation or inflammation. In addition, recent work under the NIAID contract with Utah State University has shown that T-705 has activity in rodent models against viruses other than influenza, suggesting it may have broad-spectrum activity against a range of pathogenic RNA viruses (Gowen et al., 2007).

7. Summary

The NIH and NIAID missions involve the support and conduct of research to improve the public health, both globally as well as domestically. With the combined recognition of the potential impact of emerging infections and lack of a return on investment needed for pharmaceutical drug development, the NIAID has initiated programs to try to fill in the gaps for the development of therapies with potential medical significance. The most relevant of the current programs are described here. However, some of the programs are new and all are constantly re-evaluated with additions and deletions made as deemed appropriate. Therefore the best course of action to address a developmental need for a therapy for severe viral infections, as well as for any infectious pathogen, is to contact an NIAID program person and discuss how the resources that are available might best be applied to the specific developmental challenge.

Acknowledgement

The authors would like to thank Denise Cinquegrana for excellent editorial assistance.

References

Barnard, D.L., Day, C.W., Bailey, K., Heiner, M., Montgomery, R., Lauridsen, L., Chan, P.K., Sidwell, R.W., 2006a. Evaluation of immunomodulators, interferons and known in vitro SARS-coV inhibitors for inhibition of SARS-coV replication in BALB/c mice. Antiviral Chem. Chemother. 17, 275–284.

Barnard, D.L., Day, C.W., Bailey, K., Heiner, M., Montgomery, R., Lauridsen, L., Winslow, S., Hoopes, J., Li, J.K., Lee, J., Carson, D.A., Cottam, H.B., Sidwell, R.W., 2006b. Enhancement of the infectivity of SARS-CoV in BALB/c mice by IMP dehydrogenase inhibitors, including ribavirin. Antiviral Res. 71, 53–63.

Barnard, D.L., Hubbard, V.D., Burton, J., Smee, D.F., Morrey, J.D., Otto, M.J., Sidwell, R.W., 2004. Inhibition of severe acute respiratory syndrome-associated coronavirus (SARSCoV) by calpain inhibitors and beta-D-N4-hydroxycytidine. Antiviral Chem. Chemother. 15, 15–22.

Bernstein, D.I., Harrison, C.J., 1989. Effects of the immunomodulating agent R837 on acute and latent herpes simplex virus type 2 infections. Antimicrob. Agents Chemother. 33, 1511–1515.

Bernstein, D.I., Harrison, C.J., Tomai, M.A., Miller, R.L., 2001. Daily or weekly therapy with resiquimod (R-848) reduces genital recurrences in herpes simplex virus-infected guinea pigs during and after treatment. J. Infect. Dis. 183, 844–849.

Bernstein, D.I., Stanberry, L.R., Sacks, S., Ayisi, N.K., Gong, Y.H., Ireland, J., Mumper, R.J., Holan, G., Matthews, B., McCarthy, T., Bourne, N., 2003. Evaluations of unformulated and formulated dendrimer-based microbicide candidates in mouse and guinea pig models of genital herpes. Antimicrob. Agents Chemother. 47, 3784–3788.

Bolken, T.C., Hruby, D.E., 2007. Discovery and development of antiviral drugs for biodefense: experience of a small biotechnology company. Antiviral Res..

Bonnez, W., 2005. The HPV xenograft severe combined immunodeficiency mouse model. Methods Mol. Med. 119, 203–216.

Bourne, N., Bernstein, D.I., Ireland, J., Sonderfan, A.J., Profy, A.T., Stanberry, L.R., 1999. The topical microbicide PRO 2000 protects against genital herpes infection in a mouse model. J. Infect. Dis. 180, 203–205.

Bourne, N., Bravo, F.J., Bernstein, D.I., 2000. Cyclic HPMPC is safe and effective against systemic guinea pig cytomegalovirus infection in immune compromised animals. Antiviral Res. 47, 103–109.

Bravo, F.J., Bourne, N., Schleiss, M.R., Bernstein, D.I., 2003. An animal model of neonatal cytomegalovirus infection. Antiviral Res. 60, 41–49.

Bravo, F.J., Cardin, R.C., Bernstein, D.I., 2007. A model of human cytomegalovirus infection in severe combined immunodeficient mice. Antiviral Res. 76, 104–110.

Bravo, F.J., Cardin, R.D., Bernstein, D.I., 2006. Effect of maternal treatment with cyclic HPMPC in the guinea pig model of congenital cytomegalovirus infection. J. Infect. Dis. 193, 591–597.

Buller, R.M., Owens, G., Schriewer, J., Melman, L., Beadle, J.R., Hostetler, K.Y., 2004. Efficacy of oral active ether lipid analogs of cidofovir in a lethal mousepox model. Virology 318, 474–481.

Christensen, N.D., 2005. Cottontail rabbit papillomavirus (CRPV) model system to test antiviral and immunotherapeutic strategies. Antiviral Chem. Chemother. 16, 355–362.

Christensen, N.D., Han, R., Cladel, N.M., Pickel, M.D., 2001. Combination treatment with intralesional cidofovir and viral-DNA vaccination cures large cottontail rabbit papillomavirus-induced papillomas and reduces recurrences. Antimicrob. Agents Chemother. 45, 1201–1209.

Christensen, N.D., Pickel, M.D., Budgeon, L.R., Kreider, J.W., 2000. In vivo anti-papillomavirus activity of nucleoside analogues including cidofovir on CRPV-induced rabbit papillomas. Antiviral Res. 48, 131–142.

Dow, S.W., Fradkin, L.G., Liggitt, D.H., Willson, A.P., Heath, T.D., Potter, T.A., 1999. Lipid–DNA complexes induce potent activation of innate immune responses and antitumor activity when administered intravenously. J. Immunol. 163, 1552–1561.

Furuta, Y., Takahashi, K., Fukuda, Y., Kuno, M., Kamiyama, T., Kozaki, K., Nomura, N., Egawa, H., Minami, S., Watanabe, Y., Narita, H., Shiraki, K., 2002. In vitro and in vivo activities of anti-influenza virus compound T-705. Antimicrob. Agents Chemother. 46, 977–981.

Gowen, B.B., Fairman, J., Smee, D.F., Wong, M.H., Jung, K.H., Pace, A.M., Heiner, M.L., Bailey, K.W., Dow, S.W., Sidwell, R.W., 2006a. Protective immunity against acute phleboviral infection elicited through immunostimulatory cationic liposome–DNA complexes. Antiviral Res. 69, 165–172.

Gowen, B.B., Smee, D.F., Wong, M.H., Pace, A.M., Jung, K.H., Bailey, K.W., Blatt, L.M., Sidwell, R.W., 2006b. Combinatorial ribavirin and interferon alfacon-1 therapy of acute arenaviral disease in hamsters. Antiviral Chem. Chemother. 17, 175–183.

Gowen, B.B., Wong, M.H., Jung, K.H., Sanders, A.B., Mendenhall, M., Bailey, K.W., Furuta, Y., Sidwell, R.W., 2007. In vitro and in vivo activities of T-705 against arenavirus and bunyavirus infections. Antimicrob. Agents Chemother. 51, 3168–3176.

Iyer R P, M.J., Bonnez, W., Kilkuskie, R., 2002. ORI-1001, a topical antisense oligonucleotide, for the treatment of human papillomavirus (HPV)-induced genital warts. Drugs Future 27, 546–557.

Julander, J.G., Furuta, Y., Shafer, K., Sidwell, R.W., 2007a. Activity of T-1106 in a hamster model of yellow fever virus infection. Antimicrob. Agents Chemother. 51, 1962–1966.

Julander, J.G., Morrey, J.D., Blatt, L.M., Shafer, K., Sidwell, R.W., 2007b. Comparison of the inhibitory effects of interferon alfacon-1 and ribavirin on yellow fever virus infection in a hamster model. Antiviral. Res. 73, 140–146.

Julander, J.G., Siddharthan, V., Blatt, L.M., Schafer, K., Sidwell, R.W., Morrey, J.D., 2007c. Effect of exogenous interferon and an interferon inducer on western equine encephalitis virus disease in a hamster model. Virology 360, 454–460.

Kamstock, D., Elmslie, R., Thamm, D., Dow, S., 2007. Evaluation of a xenogeneic VEGF vaccine in dogs with soft tissue sarcoma. Cancer Immunol. Immunother. 56, 1299–1309.

Kern, E.R., Bidanset, D.J., Hartline, C.B., Yan, Z., Zemlicka, J., Quenelle, D.C., 2004a. Oral activity of a methylenecyclopropane analog, cyclopropavir, in animal models for cytomegalovirus infections. Antimicrob. Agents Chemother. 48, 4745–4753.

Kern, E.R., Collins, D.J., Wan, W.B., Beadle, J.R., Hostetler, K.Y., Quenelle, D.C., 2004b. Oral treatment of murine cytomegalovirus infections with ether lipid esters of cidofovir. Antimicrob. Agents Chemother. 48, 3516–3522.

Kocisko, D.A., Caughey, B., Morrey, J.D., Race, R.E., 2006. Enhanced antiscrapie effect using combination drug treatment. Antimicrob. Agents Chemother. 50, 3447–3449.

Milligan, G.N., Bernstein, D.I., 1997. Interferon-gamma enhances resolution of herpes simplex virus type 2 infection of the murine genital tract. Virology 229, 259–268.

Morrey, J.D., Day, C.W., Julander, J.G., Blatt, L.M., Smee, D.F., Sidwell, R.W., 2004a. Effect of interferon-alpha and interferon-inducers on West Nile virus in mouse and hamster animal models. Antiviral Chem. Chemother. 15, 101–109.

Morrey, J.D., Day, C.W., Julander, J.G., Olsen, A.L., Sidwell, R.W., Cheney, C.D., Blatt, L.M., 2004b. Modeling hamsters for evaluating West Nile virus therapies. Antiviral Res. 63, 41–50.

Morrey, J.D., Siddharthan, V., Olsen, A.L., Roper, G.Y., Wang, H., Baldwin, T.J., Koenig, S., Johnson, S., Nordstrom, J.L., Diamond, M.S., 2006. Humanized monoclonal antibody against West Nile virus envelope protein administered after neuronal infection protects against lethal encephalitis in hamsters. J. Infect. Dis. 194, 1300–1308.

Morrey, J.D., Siddharthan, V., Olsen, A.L., Wang, H., Julander, J.G., Hall, J.O., Li, H., Nordstrom, J.L., Koenig, S., Johnson, S., Diamond, M.S., 2007. Defining limits of treatment with humanized neutralizing monoclonal antibody for West Nile virus neurological infection in a hamster model. Antimicrob. Agents Chemother. 51, 2396–2402.

Morrey, J.D., Smee, D.F., Sidwell, R.W., Tseng, C., 2002. Identification of active antiviral compounds against a New York isolate of West Nile virus. Antiviral Res. 55, 107–116.

Noah, J.W., Severson, W., Noah, D.L., Rasmussen, L., White, E.L., Jonsson, C.B., 2007. A cell-based luminescence assay is effective for high-throughput screening of potential influenza antivirals. Antiviral Res. 73, 50–59.

Parker, S., Touchette, E., Oberle, C., Almond, M., Robertson, A., Trost, L.C., Lampert, B., Painter, G., Buller, R.M., 2008. Efficacy of therapuetic intervention with an oral ether lipid analogue of cidofovir (CMX001) in a lethal mousepox model. Antiviral Res. 77, 39–49.

Prichard, M.N., Keith, K.A., Quenelle, D.C., Kern, E.R., 2006. Activity and mechanism of action of *N*-methanocarbathymidine against herpesvirus and orthopoxvirus infections. Antimicrob. Agents Chemother. 50, 1336–1341.

Quenelle, D.C., Buller, R.M., Parker, S., Keith, K.A., Hruby, D.E., Jordan, R., Kern, E.R., 2007. Efficacy of delayed treatment with ST-246 given orally against systemic orthopoxvirus infections in mice. Antimicrob. Agents Chemother. 51, 689–695.

Quenelle, D.C., Collins, D.J., Kern, E.R., 2003. Efficacy of multiple- or single-dose cidofovir against vaccinia and cowpox virus infections in mice. Antimicrob. Agents Chemother. 47, 3275–3280.

Quenelle, D.C., Collins, D.J., Kern, E.R., 2004a. Cutaneous infections of mice with vaccinia or cowpox viruses and efficacy of cidofovir. Antiviral Res. 63, 33–40.

Quenelle, D.C., Collins, D.J., Marciani, D.J., Kern, E.R., 2006a. Effect of immunization with herpes simplex virus type-1 (HSV-1) glycoprotein D (gD) plus the immune enhancer GPI-0100 on infection with HSV-1 or HSV-2. Vaccine 24, 1515–1522.

Quenelle, D.C., Collins, D.J., Wan, W.B., Beadle, J.R., Hostetler, K.Y., Kern, E.R., 2004b. Oral treatment of cowpox and vaccinia virus infections in mice with ether lipid esters of cidofovir. Antimicrob. Agents Chemother. 48, 404–412.

Quenelle, D.C., Keith, K.A., Kern, E.R., 2006b. In vitro and in vivo evaluation of isatin-beta-thiosemicarbazone and marboran against vaccinia and cowpox virus infections. Antiviral Res. 71, 24–30.

Samuel, M.A., Wang, H., Siddharthan, V., Morrey, J.D., Diamond, M.S., 2007. Axonal transport mediates West Nile Virus entry into the central nervous system and induces acute flaccid paralysis. Proc. Natl. Acad. Sci. U. S. A. 104, 17140–17145.

Schriewer, J., Buller, R.M., Owens, G., 2004. Mouse models for studying orthopoxvirus respiratory infections. Methods Mol. Biol. 269, 289–308.

Severson, W.E., Shindo, N., Sosa, M., Fletcher IIIrd, T., White, E.L., Ananthan, S., Jonsson, C.B., 2007. Development and validation of a high-throughput screen for inhibitors of SARS CoV and its application in screening of a 100,000-compound library. J. Biomol. Screen 12, 33–40.

Sidwell, R.W., Barnard, D.L., Day, C.W., Smee, D.F., Bailey, K.W., Wong, M.H., Morrey, J.D., Furuta, Y., 2007. Efficacy of orally administered T-705 on lethal avian influenza A (H5N1) virus infections in mice. Antimicrob. Agents Chemother. 51, 845–851.

Smee, D.F., Hurst, B.L., Wong, M.H., Glazer, R.I., Rahman, A., Sidwell, R.W., 2007a. Efficacy of *N*-methanocarbathymidine in treating mice infected intranasally with the IHD and WR strains of vaccinia virus. Antiviral Res. 76, 124–129.

Smee, D.F., Morrison, A.C., Barnard, D.L., Sidwell, R.W., 2002. Comparison of colorimetric, fluorometric, and visual methods for determining anti-influenza (H1N1 and H3N2) virus activities and toxicities of compounds. J. Virol. Methods 106, 71–79.

Smee, D.F., Wandersee, M.K., Bailey, K.W., Wong, M.H., Chu, C.K., Gadthula, S., Sidwell, R.W., 2007b. Cell line dependency for antiviral activity and in vivo efficacy of *N*-methanocarbathymidine against orthopoxvirus infections in mice. Antiviral Res. 73, 69–77.

Smee, D.F., Wandersee, M.K., Wong, M.H., Bailey, K.W., Sidwell, R.W., 2004a. Treatment of mannan-enhanced influenza B virus infections in mice with oseltamivir, ribavirin and viramidine. Antiviral Chem. Chemother. 15, 261–268.

Smee, D.F., Wong, M.H., Bailey, K.W., Beadle, J.R., Hostetler, K.Y., Sidwell, R.W., 2004b. Effects of four antiviral substances on lethal vaccinia virus (IHD strain) respiratory infections in mice. Int. J. Antimicrob. Agents 23, 430–437.

Smee, D.F., Wong, M.H., Bailey, K.W., Sidwell, R.W., 2006. Activities of oseltamivir and ribavirin used alone and in combination against infections in mice with recent isolates of influenza A (H1N1) and B viruses. Antiviral Chem. Chemother. 17, 185–192.

Stanberry, L.R., Harrison, C.J., Bravo, F.J., Childs, F., Reece, A.L., Bernstein, D.I., 1990. Recurrent genital herpes in the guinea pig augmented by ultraviolet irradiation: effects of treatment with acyclovir. Antiviral Res. 13, 227–235.

Takahashi, K., Furuta, Y., Fukuda, Y., Kuno, M., Kamiyama, T., Kozaki, K., Nomura, N., Egawa, H., Minami, S., Shiraki, K., 2003. In vitro and in vivo activities of T-705 and oseltamivir against influenza virus. Antiviral Chem. Chemother. 14, 235–241.

U'Ren, L.W., Biller, B.J., Elmslie, R.E., Thamm, D.H., Dow, S.W., 2007. Evaluation of a novel tumor vaccine in dogs with hemangiosarcoma. J. Vet. Intern. Med. 21, 113–120.

Wyde, P.R., Chetty, S.N., Jewell, A.M., Schoonover, S.L., Piedra, P.A., 2005a. Development of a cotton rat-human metapneumovirus (hMPV) model for identifying and evaluating potential hMPV antivirals and vaccines. Antiviral Res. 66, 57–66.

Wyde, P.R., Chetty, S.N., Timmerman, P., Gilbert, B.E., Andries, K., 2003. Short duration aerosols of JNJ 2408068 (R170591) administered prophylactically or therapeutically protect cotton rats from experimental respiratory syncytial virus infection. Antiviral Res. 60, 221–231.

Wyde, P.R., Laquerre, S., Chetty, S.N., Gilbert, B.E., Nitz, T.J., Pevear, D.C., 2005b. Antiviral efficacy of VP14637 against respiratory syncytial virus in vitro and in cotton rats following delivery by small droplet aerosol. Antiviral Res. 68, 18–26.

Wyde, P.R., Moore-Poveda, D.K., De Clercq, E., Neyts, J., Matsuda, A., Minakawa, N., Guzman, E., Gilbert, B.E., 2000a. Use of cotton rats to evaluate the efficacy of antivirals in treatment of measles virus infections. Antimicrob. Agents Chemother. 44, 1146–1152.

Wyde, P.R., Stittelaar, K.J., Osterhaus, A.D., Guzman, E., Gilbert, B.E., 2000b. Use of cotton rats for preclinical evaluation of measles vaccines. Vaccine 19, 42–53.