INA RESPOND
INDONESIA RESEARCH PARTNERSHIP ON INFECTIOUS DISEASE

Newsletter
April 2017

INA-RESPOND Secretariat

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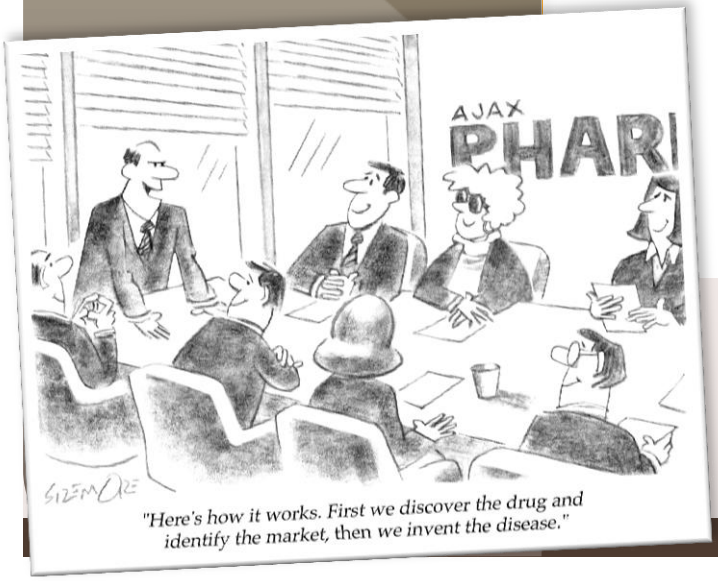
In This Issue

INA-RESPOND to Eradicate Schistosomiasis in Indonesia

During the courtesy visit from Dr Clifford Lane on March, 10th, 2017, dr. Untung Suseno Sutarjo, M.Kes, the general secretary of Ministry of Health, Indonesia, requested INA-RESPOND to conduct studies related with the eradication of Shistosomiasis.

Schistosomiasis is an acute and chronic disease caused by Schistostoma, parasitic flatworms belong to a genus of trematodes, commonly known as blood-flukes. Based on the egg morphology, intermediate host-specificity and geographic regions this genus is divided into four groups — *indicum*, *haematobium*, *mansoni*, and japonicum. The last group consists of three species, *S. japonicum*, *S. malayensis* and *S. mekongi*.

Read the complete report on page 5



Swear to God, I Don't Have Any Competing Interests!

Conflict of interests/competing interests is not an issue to be taken lightly especially in the scientific world. Everyone has risk of being caught up in competing interests. Read our comic corner and find out if you are exposed to them.

Save The Date

Important Events & Meetings

14 April	Good Friday (Religious holiday)
24 April	Isra Mi'raj Nabi Muhammad SAW (Religious holiday)

April Birthday

4 Apr	Hofiya Djauhari, Msi.	INA101 Lab Tech Site 510
6 Apr	dr. Heni Kismayawati	NIHRD
11 Apr	dr Tutik Kusmiati, SpP(K)	INA102 Site PI Site 570
13 Apr	dr Haviv Muris Saputra	INA101 RA Site 570
22 Apr	dr. M.M.D.E.A.H. Hapsari	INA101 Co-PI Site 560
27 Apr	Prof dr Emiliana Tjitra	NIHRD

Announcement

Congratulations to dr. Fatmawati Ahmad (INA101 RA, Site 550) for your new born baby; Wishing you happy moments with your little angel. Also, congratulations to dr. Debby Permatasari (INA101 RA, Site 540) for your wedding. Much love, health, and happiness to both you and your husband.

By:
dr. Nurhayati
dr. Anandika Pawitri

AFIRE Study (INA101) Updates

As stated in the protocol, the main objective of this study is to identify the etiology of acute febrile illness cases. Up to March 2017, approximately 65% of the cases had been identified based on standard of care at the site hospitals [blood culture or other specimen cultures (e.g. urine, pus)] and by the study. The results were presented to the Protocol PIs and Steering Committee members during NSC meeting on 9 March 2017.

To disseminate our preliminary data, the investigators were grouped to prepare manuscripts based on their interests and expertise. We are also preparing some abstracts for several international meetings.

Detailed screening and enrollment progress is available in portal folder: Studies\INA101\Screening progress.pdf or go to the following link: https://ina-respond.net/EdmFile/getfile/797233
For further information about this study please go to: http://www.ina-respond.net/afire-study/

TRIPOD (INA102) Updates

Site activation

Sanglah Hospital in Denpasar was our first active TRIPOD site. Since then, 2 sites (dr Kariadi Hospital, Semarang and dr Soetomo Hospital, Surabaya) have also started screening and enrollment activities. At first, Sanglah Hospital made a rather slow progress on enrolling patient, but now they are catching up with the weekly enrollment target. The sites agree to start with 1 patient/week so they can maintain the quality of the data and CRF.

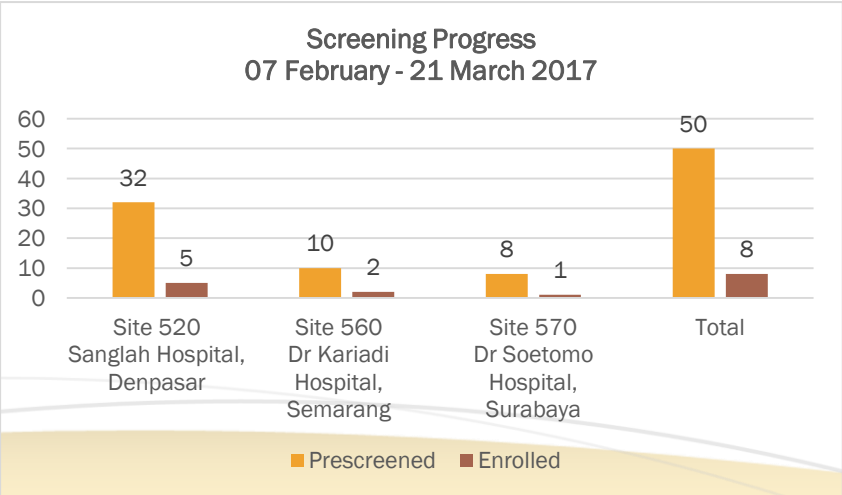
Dr Sardjito Hospital, Yogyakarta started to enroll subject on 29 March 2017 after socializing the study to other related hospital's staff. Next sites that will be activated are Hasan Sadikin Hospital, Bandung and Wahidin Sudirohusodo Hospital, Makassar.

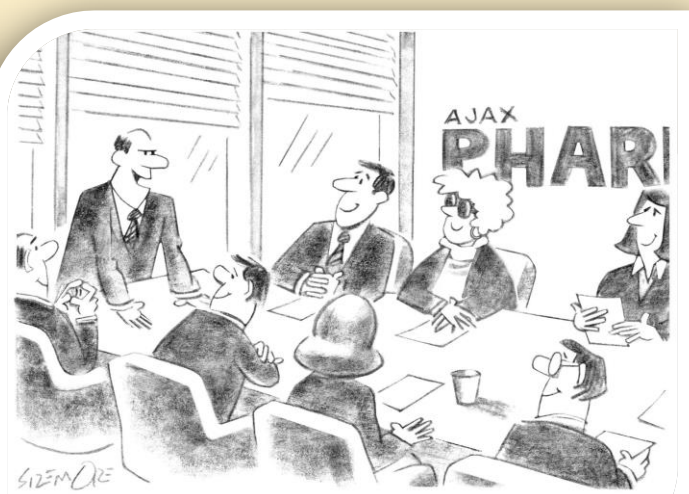
	Site 520	Site 560	Site 570
1st subject enrolled	7 Feb 2017	1 Mar 2017	7 Mar 2017

Screening progress report

Around 16% of the pre-screened patients have agreed to join the study. The most-common reasons why patients cannot join the study are:

- Other (patient refuses to join the study, delirium, poor general condition, weekly enrollment target achieved)
- Patients have already received TB treatment in the last 2 month
- Non-suggestive TB x-ray





"Here's how it works. First we discover the drug and identify the market, then we invent the disease."

Comic Corner:

Swear to God, I don't have any competing interests!

By:

dr. Aly Diana

As researchers and authors, we have to admit that most of the funding sources for research come from organization/enterprise/industry which may influence the way we develop the study design, data collection, analysis, and interpretation, writing of the paper, and/or decision to submit for publication. In other words, conflict of interests is mostly unavoidable. Therefore, every time we want to do a submission in the journals, there is usually a part that specifically highlights about funding sources and conflict or competing interests. The sentence in the article stating that "We declare no conflict of interest" or "None of the authors had a personal or financial conflict of interest" actually has a deeper meaning than we may think.

We have to realize that public trust in the scientific process and the credibility of published articles depend in part on how transparently conflicts of interest are handled during the planning, implementation, writing, peer review, editing, and publication of scientific work. By knowing the full scopes of the things that can be called as conflict of interests, we can give a more confident disclosure that we really don't have any conflict of interest to declare. Or if we do have competing interest, we can describe it confidently as well. Please remember that failure to declare competing interests can result in immediate rejection of a manuscript; and if an undisclosed competing interest comes to light after publication, journals most likely will issue a public notification to the community.

Conflict of interest is not only for authors, but also for editors, reviewers, and readers who comment on published articles. Competing interests can be financial or non-financial, professional or personal; and can arise in relationship to an organization or another person. Although non-financial competing interests may not be as popular as financial ones, there is a growing concern about them. Like financial interests, they can influence personal judgement. For example, what do you think will happen to a paper that discussed a new drug for abortion - when it is being reviewed by an editor who totally opposes abortion practice in general? Do you think that the chance of the paper to get published will be different if it is assigned to be reviewed by another editor who has a neutral background?

Sadly to say, like all human activity, academic research and scientific publishing are not free from subjectivity, imperfection, and may be prone to bias, corruption, and self-interest. In addition, professional affinities and rivalries, nepotism, scientific competition, religious beliefs, and political or ideological views are often lead to unfair judgments; private competing interests are perhaps even more potent than financial ones.

Another interesting fact was found by Goldsmith and colleagues who reviewed 228 consecutive manuscripts submitted to the *Journal of Investigative Dermatology* in 2003: the odds of acceptance is two times higher for manuscripts from which authors had excluded reviewers, compared to those whose authors had not done so. In conclusion for his study, it can be said "Excluding reviewers ends up being very, very important. People know their assassins." Again, this example is given to give a sense on how non-financial competing interests may negatively/positively affect the publication of the paper.

Closing remarks: Yes, conflict of interests/competing interests is a big deal in the real science world. It's not only for authors, but also for editors/reviewers. It's not only about financial, but also about non-financial (personal) competing interests. Science is based on trust and that's our responsibility to earn that trust.

INA-RESPOND to Eradicate Schistosomiasis in Indonesia

By:

dr. Herman Kosasih

During the courtesy visit from Dr Clifford Lane on March, 10th, 2017, dr. Untung Suseno Sutarjo, M.Kes, the general secretary of Ministry of Health, Indonesia, requested INA-RESPOND to conduct studies related with the eradication of Shistosomiasis.

Schistosomiasis is an acute and chronic disease caused by Schistostoma, parasitic flatworms belong to a genus of trematodes, commonly known as blood-flukes. Based on the egg morphology, intermediate host-specificity and geographic regions this genus is divided into four groups — *indicum*, *haematobium*, *mansoni*, and japonicum. The last group consists of three species, *S. japonicum*, *S. malayensis* and *S. mekongi*.

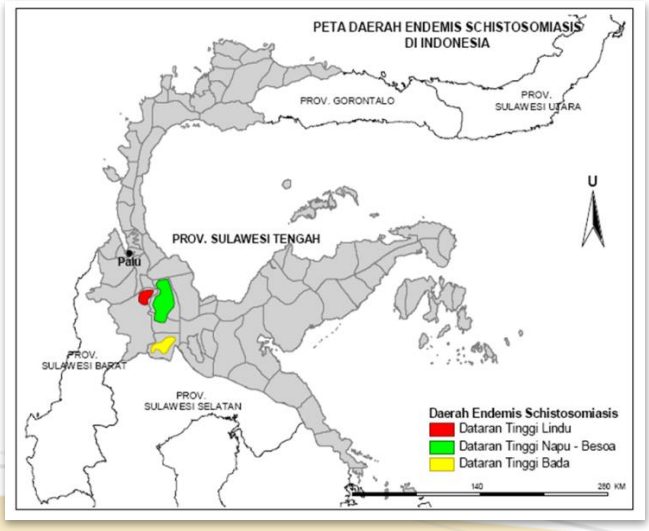
Schistosomiasis has three clinical states related to the life cycle: the first stage is characterized by dermatitis and is caused by the cercariae, the second stage is marked by fever and constitutional complaints (Katayama fever), and the third stage results in chronic fibro-obstructive disease which is caused by the trapped eggs in the liver.

WHO estimates that there are more than 200 million people needed preventive treatment in 2015 and about 200 000 deaths globally each year due to chronic schistosomiasis.

In Indonesia, schistosomiasis was first reported in Lindu in 1937, followed by Napu in 1972 and Bada in 2008. All is caused by Schistostoma japonicum with snail (*Oncomelania hupensis lindoensis*) as the intermediate host. (Figure 1)

Although several efforts such as schistosomiasis research had been studied since 1940, an invasive schistosomiasis control was initiated in 1982 and an inter-sectoral project (Central Sulawesi Integrated Area Development and Conservative Project) was conducted in 1995-2005, the proportion of schistosomiasis in humans remains at 0.8% in Lindu and at 1.4% in Napu highland in 2012, and the infection rates in snails and in rodents in Lindu were 1.8% and 16%, respectively and in Napu were 1.1% and 7.3%, respectively.

INA-RESPOND can assist the local government eliminate schistosomiasis in these three endemic areas by conducting a comprehensive study including: 1) how to diagnose schistosoma infection at all stages accurately using molecular and serological assays in comparison to the gold standard; 2) to estimate the prevalence of disease in these areas, and 3) to compare the efficacy of praziquantel 40mg/kg body weight, single dose, with other regimens (e.g. higher dose of praziquantel, and the combination of praziquantel and artesunate derivates).



Report:

HIV and Chimeric Antigen Receptors for Cancer Treatment

By:

dr. Dona Arlinda

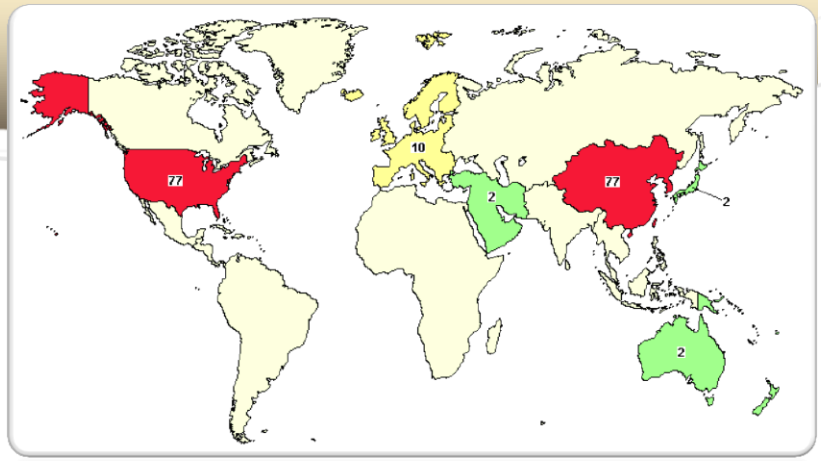


Fig 1 Country Distribution of Clinical Trials on Chimeric Antigen Receptors in Cancer

Alongside with the advancement of personalised medicine, anticancer drug development is currently directed towards targeted therapies. While standard cancer chemotherapies kill both fast-growing cancer cells and the healthy ones, targeted cancer therapies work with specific molecular targets that are involved in the growth, progression, or spread of cancer. There are many types of targeted therapies that have been approved for cancer treatment, such as hormone therapies, signal transduction inhibitors, gene expression modulators, apoptosis inducers, angiogenesis inhibitors, immunotherapies, and toxin delivery molecules. Immunotherapies in particular, gained much attention at the Conference on Retroviruses and Opportunistic Infections (CROI) 2017. Monoclonal antibodies can be used to recognize specific molecules on the surface of cancer cells and subsequently kill them. Another usage involves linking monoclonal antibodies to other immune cells in order to help them kill cancer cells.

Dr. Carl H. June from the University of Pennsylvania presented the use of targeted immunotherapy using Chimeric Antigen Receptors Lymphocyte T-cells (CAR T-cells) for cancer treatment. CAR T-cells consist of antigen-binding region of a monoclonal antibody linked to intracellular T-cell signalling domains. CAR T-cells have antibody specificity that exert cytotoxic effects when bind to their target. In this case, Dr. June used CD19, a tumour-specific antigen found in normal and malignant B cells and B-cell precursors (CART19).

Although chimeric antigen receptors-based therapies are relatively new, clinically significant antitumor activity is shown in neuroblastoma, chronic lymphocytic leukemia, and B-cell lymphoma, as well as other adult and paediatric malignancies. Currently, China and the USA are two leading countries with clinical trials on chimeric antigen receptors in cancer (Fig. 1).

Dr. June and his team first used CART19 in 2012 on a 7-year-old girl with relapsed and refractory pre–B-cells acute lymphoblastic leukemia (ALL). Dr June initially took out the patient' own lymphocyte T-cells and fused them together with HIV viruses to induce the expression of chimeric antigen receptors. The CAR T-cells were designed to target CD19 molecules on the cancer cells. The CART19 cells were then grown in the laboratory and were infused back to the patient.

At first, the patient experienced severe toxic effects consistent with cytokine-release syndrome, which manifested as hypotension, acute vascular leak syndrome, and acute respiratory distress syndrome that required intubation. Laboratory findings include elevated levels of ferritin, triglycerides, aminotransferases, bilirubin (primarily conjugated), and soluble interleukin-2 receptor a-chain and decreased levels of fibrinogen. This cytokine-release syndrome appeared to be reversible and was rapidly cleared following the administration of anticytokine therapy. After 180 days of CART19 treatment, the patient came out cancer free and has remained so

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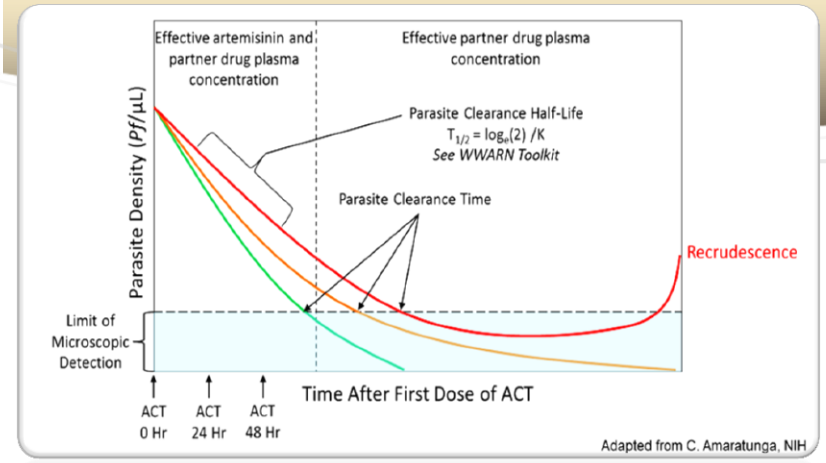

Picture 1 : Understanding Treatment Failure

Clinical and Molecular Studies of Drug-Resistant *Plasmodium Falciparum* in Southeast Asia

By:

dr. Patricia Tauran

On 6-7 March, RSU Tangerang hospital held a malaria workshop for trainers. The workshop invited several malaria experts to speak to the participants: Prof. Dr. Ida Parwati, DR. Aaron Neal, and Mr. Awalludin Sutamihardja.

Indonesia, as one of the countries located in South East Asia is really interested in malaria drug resistance, which often becomes an issue in South East Asia countries. The following is the summary of one of the speakers' presentations in the workshop.

Modern malaria treatment → Wide range of treatment options exist

Antimalarial Mechanisms:

- Nearly all antimalarial acts on hemoglobin digestion pathways
  - Parasites must detoxify the heme in hemoglobin, which is done by forming stable crystals of hemozoin
  - Partially explains why resistance against entire drug families, such as quinolones, can develop
- Artemisinin is thought to generate free radicals or reactive oxygen species
  - Would cause broad, non-specific cellular damage
  - New classes of antimalarial are being developed with free radical generation in mind

Use is limited by various factors:

- Availability and/or country treatment guidelines
- Patient genetics such as glucose 6-phosphate dehydrogenase (G6PD) deficiency
- Widespread resistance

Artemisinin was introduced in 1971 and became the safest and fastest acting antimalarial in history. Combination of drugs overcomes short plasma half-life of artemisinin and minimizes potential for parasites to develop resistance to either drug

Southeast Asia (SEA) is consistently the site of emerging *P. falciparum* drug resistance

Artemisinin resistance is widespread in SEA

- Clinic detects by blood smear and parasite clearance time

Blood smear

Patients with slow-clearing infections frequently present with "dormant" ring stage parasites.

Slow-clearing isolates appear to have a prolonged ring stage:

- Ring stage is less susceptible to DHA
- Possibly driven by a genetic mutation

Parasite clearance time

The success of ACT treatment is determined by measuring the

Class	Drugs
4-Aminoquinoline	Chloroquine, Amodiaquine, Piperaquine
8-Aminoquinoline	Primaquine, Quinine
Arylamino alcohol	Mefloquine, Lumefantrine
Sesquiterpene lactone endoperoxides	Artemether, Artesunate, Dihydroartemisinin
Mannich base	Pyronaridine
Antifolate	Sulfadoxine/Pyrimethamine
Naphthoquinone/antifolate	Atovaquone/Proguanil
Antibiotic	Doxycycline, Clindamycin

Green – susceptible, red – resistant, black – resistant but still in research

parasite clearance time or parasite clearance half-life. (See picture 1)

- Lab detects by IC_{50}, RSA and Kelch-13 gene sequencing

  IC_{50} values were an imperfect measure of delayed parasite clearance. An alternative to the IC_{50} assay, a physiologically-relevant *in vitro* drug assay was developed called the Ring-stage Survival Assay (RSA). RSA mimics the short plasma half-life of artemisinin (DHA). RSA spans 72 hours so that viable trophozoites can be seen easily by microscopy. RSA requires 2 trained microscopists reading slides in triplicate. Flow cytometry offers high-throughput, highly accurate data collection within 24 hours

Early Signs of ACT Failure → delayed parasite clearance times were observed in patients from Cambodia but not Thailand in 2007-8.
Further Signs of ACT Failure → Elevated parasite clearance half-life values were seen in a second Cambodian province during 2009-2010

TRAC (Tracking Resistance to Artemisinin Collaboration) Study (10 countries, 15 sites), from 2011-2013, enrolled 1241 adults and children with acute, uncomplicated falciparum malaria, determined parasite clearance half-lives and mapped the spread of artemisinin resistance.

Searching for Mutations

- Many methods were applied to slow- and fast-clearing parasites to find the causal mutation(s):
  - Full genome sequencing and genome wide association studies (GWAS)
  - *in vitro* genetic crosses and linkage analysis
  - RNA sequencing and proteomics
- Ultimately, a brute-force drug pressure study revealed what was likely the responsible mutation

A Gene of Interest

- Over 5 years, an artemisinin-sensitive isolate was continuously cultured under cycling drug pressure. Eventually, a non-synonymous SNP appeared in gene *Pf3D7_1343700*. Slow-clearing isolates were then sequenced and all had SNPs in the same gene
- Additional SNPs were observed but did not fully correlate with field isolates
- Later experiments showed that the relationship between *Pf3D7_1343700* and decreased artemisinin susceptibility is indeed causal. Interestingly, the level of resistance seems to change depending on the specific mutation in *Pf3D7_1343700*

Kelch-13 Protein

- *Pf3D7_1343700* had not been previously described. It shares high sequence homology with multi-domain human Kelch proteins
- Kelch proteins generally recognize and respond to oxidants and other cell stressors. Kelch-13 (K13) has 6 propeller blades that likely function as a binding surface. All causal mutations are found on these propellers. Over 100 mutations have been seen in field isolates to date.

Piperaquine resistance is now a concern in SEA

- Clinic detects by blood smear and parasite clearance time
- Lab detects by IC_{50} assay and exo-E415G/Plasmepsin 2-3 sequencing

Piperaquine and mefloquine resistance may be mutually exclusive

- Treatment using Artemisin-based Combination Therapy takes advantage of this to halt resistance spreading.

Malaria Training of Teacher Workshop on 6-7 March 2017 at RSU Tangerang.

Speakers: Prof. Dr. Ida Parwati, DR. Aaron Neal, and Mr. Awalludin Sutamihardja

Continued from page 6

for five consecutive years.

After the success of the first patient, they continue to CART19 phase 1 clinical trial involving 60 pediatrics/young adults ALL patients which showed 93% complete remission rate. These remarkable results were submitted to the FDA and they anticipated approval in 2017.

In summary, CART19 is a fine example of engineering T-cells for cancer therapy. Although at some point HIV viruses were used as vectors to introduce the chimeric antigen receptors, Dr. June was convinced that the virus would not be able to infect the patient or cause disease. The one thing that he was not sure at that time was whether this treatment could cure the cancer itself. We look forward to the results of bigger and well defined cohort studies of CART19 in the near future.

Selected references:

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INA-RESPOND Newsletter

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Thanks to : INA-RESPOND Network and Partners

We would like to hear from you. Go ahead and send us your scientific articles, team profile, or feedback to INA.Secretariat@ina-respond.net

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