International SAE Consortium
Phase 2 Drug Induced Serious Adverse Event Research Summary
December 2009- December 2012

INTRODUCTION

The Serious Adverse Event Consortium (SAEC) is a pharmaceutical industry (10 members) led biomedical research consortium (*the Wellcome Trust and PGx Health are associate members*), focused on identifying DNA-variants potentially useful in predicting the risk and understanding the mechanisms of drug induced serious adverse events. Specifically, the SAEC coordinates and develops SAE research networks to obtain well phenotyped SAE cases and controls for its research endeavors. It collaborates across the pharmaceutical industry, and with providers and leading academic researchers to apply whole genome genotyping and sequencing technology to discover and confirm genetic markers associated with SAEs. As a vital component of its commitment to enabling and facilitating this important research, the SAEC releases "allowable" research data (*i.e. as approved by IRBs and specified in the relevant informed consents*) to qualified researchers via the SAEC's website (www.saeconsortium.org).

The SAEC's value to its membership and to the public are large derive primary from four benefits:

1. First and foremost, through its sponsored research (*which is at a scale beyond any one entity's capabilities*) it is providing novel insight into the genetic basis of the drug induced serious adverse events (SAEs) most likely to cause a drug to fail in development or the marketplace. It is providing access to all its raw data (genotype and phenotype), and additional data contributed by member companies, to all researchers free of charge. Overtime as the breadth and depth of the SAEC's research and database grow, it is hoped this knowledge and data will reduce product development failures and improve the safety profile of compounds in pre-clinical and clinical development. This could be worth billions in value over the long term. The SAEC has modeled, over a 15 year period, the economic value (NPV) potentially associated with reduced product development failures, reduced product withdrawals, and improved healthcare productivity could approach $US20 billion. Furthermore, all of the "genetic variants" discovered by the SAEC are being filed with the international patent offices, using a method that ensures the earliest possible date of discovery and leaves them forever unencumbered by IP constraints.
2. The SAEC provides its members a with unique and high quality public relations benefit. At a time when many in government and the press are all too willing to talk down the pharmaceutical industry, the SAEC is a wonderful example of private sector leadership/initiative driven by the industry, with great potential public health and research benefit. Opportunities for this level and quality of recognition are very valuable to all members.
3. The SAEC provides a unique opportunity for members to partner with our regulatory colleagues to develop and learn (together) how to apply the methods and findings of

safety pharmaco-genetic research. The SAEC enhances members' relationship with the FDA in general and CDER specifically, as well as with international regulatory bodies such as PMDA and EMEA. The first phase of the SAEC has been deemed by the FDA as, "An extraordinary and a glowing private sector leadership example; a model for how a private sector biomedical consortium should effectively operate". This reflects well on all of the SAEC's members.

4. Finally, the SAEC provides its membership with novel opportunities to develop its relationship with major health systems/providers (*e.g. HMO Research Network*). By working together to research and apply genetics to improve the safe use of drugs, each member is enhancing its long term relationship and reputation with some of its most important customers. These relations should afford members, over time, with new channels to research both the safety and efficacy of their products.

Although the Consortium's scientific scope is broad in principle, it has focused during its first phase (*September 2007-December 2009*) on two initial projects: Identifying genetic variants associated with drug-induced liver injury (DILI) and serious skin rash (Stevens - Johnson syndrome (SJS) and Toxic Epidermal Necrolysis (TEN)). These two projects have allowed the SAEC to generate initial results in a relatively short time frame (*due to the availability of established case-control DNA sample collections and only 12 months of additional SAE case ascertainment in the case of the DILI research*).

PHASE 1: Primary objectives and results

The primary objectives of the **SSR** project were:

- To serve as a pilot study for establishing the DACC, implementation and testing of hardware infrastructure, data management, analysis process and reporting. **This objective was achieved.**
- To identify potential genetic marker(s) that confer an increased risk of SSR of a modest to large effect size (i.e. *which would be observable in relatively small number (~75) of patients*). **This objective was achieved.**

The methodology of the **SSR** project was:

- Focus on Caucasian cases only (76 cases)
- Use the Illumina 1M chip to genotype ~250 country and sex-matched population controls (shared in common with the DILI project), and
- Draw on publicly available Wellcome Trust Case Control Consortium (WTCCC) for (~5,000) matched controls with the aim of increasing the power to detect associated DNA variants.

The primary results of the **SSR** project were:

- The 3 most associated SNPs, ($P < 10^{-7}$) were close to or within biologically-relevant genes on chromosomes 6 and 7, and require replication in an independent data set
- By using larger numbers of population controls (100 controls for each case) from the WTCCC, we improved the power of the study.
- In phase 2, we plan to add significantly to the case cohort to strengthen these findings, and to explore genetic differences across ethnicities and drug types.

The primary objectives of the **DILI** project were:

- To establish an international network to study the genetics of DILI. **This objective was achieved.**
- To identify potential genetic marker(s) of genome wide significance (i.e. *which would be observable in relatively small numbers of patients*). **This objective was achieved.**

The methodology of the **DILI** project was:

- Focus on the Caucasian cases only (500 cases),
- Use the Illumina 1M chip genotyped ~750 country and sex-matched population controls (shared with the SSR project),
- Draw on publicly available WTCCC for additional genetically-matched controls, and
- Complete HLA genotyping (class I & II) on a subset of the flucloxacillin and all of the coamoxiclav DILI cases.

The primary results (to date) of the **DILI** project are:

- *HLA-B*5701* was discovered as a major risk factor of liver injury due to flucloxacillin
- HLA genes are major genetic factors of coamoxiclav liver injury
    - The previous report of an association of DR2 (*HLA-DRB1*1501-DQA1*0102-DQB1*0602*) was confirmed through a tag SNP (rs3135388)
    - It also generated novel findings:
        - Fine mapping at MHC class II rejects the previously-suspected DR2 as the sole risk factor
        - Another marker at MHC class II is most-associated; it suggests the association of *DRB1*0801-DQA1*0401-DQB1*0402* in addition to DR2
        - An independent risk allele at MHC class I: *HLA-A*0201*.
- We also expanded the DILI cases by adding in 70 (Abbott) Zileuton DILI Caucasian cases. Initial analyses display genome-wide significant SNPs on chromosome 2.
- No robustly associated SNPs were identified in DILI across all drugs, nor have any been validated due to drugs excluding flucloxacillin and coamoxiclav. This is likely due to cohort size limitations to detect modest, and in some cases major, effects.

PHASE 1: Additional achievements

In addition to the aforementioned, the SAEC has also generated the following results in phase one, which have helped to lay the framework for its phase two plan:

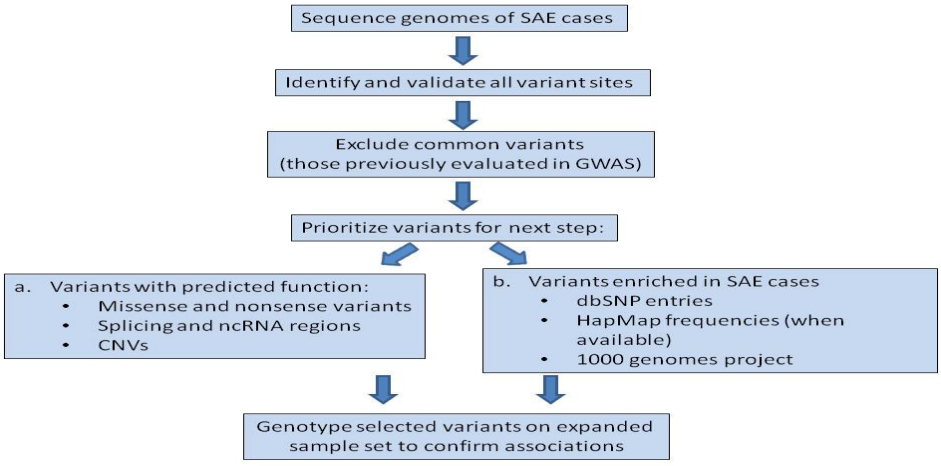
- We have assembled one of the largest DILI research collections in the world, generating significant GWAS results and three publications (*Nature Genetics article published 6/09, two in preparation)*
- We have organized four new SAE GWAS studies with minimal investment (*Abbott DILI, DARE prolonged QT/torsades de pointes, angio-edema, and clozapine agranulocytosis*) and **2** whole genome sequencing pilots (*agranulocytosis and DILI*)

- It filed provisional patent applications (5) to ensure a "clear date of discovery" on SSR and DILI markers, in support of its public data releases
- It created and launched a publicly available "knowledge base" to identify PGx markers involved in key SAEs, via a dedicated SAEC web-site (*SSR 1, DILI 1, POPRES 1, and unplanned GWAS & WG studies*)
- It initiated a major SAE phenotyping standardization effort to facilitate both SAE surveillance and 3 pilots for SAE research via electronic medical records (*VA, HMORN & Cerner, Inc.*)
- Finally, it laid the ground work for developing (Phase 2) global academic networks for expansion of SSR, DILI, and Acute Hypersensitivity Reaction cohorts … while enabling the precedent of member contributions of SAE cases (*e.g. Abbott, GSK, & Sanofi-Aventis*)

Transition to PHASE 2

The phase 1 work has made it clear that the common variants interrogated in GWAS studies are likely to explain only a proportion of the increased risk of an adverse event in susceptible individuals. The SAEC is therefore taking advantage of emerging next generation sequencing technologies to explore the role of rare genetic variants (minor allele frequency < 1%) in drug induced SAEs, using next generation whole-genome sequencing.

The first study, being carried out in conjunction with Duke University, will sequence at high coverage the entire genomes of 20 patients with clozapine-induced agranulocytosis. In this study, due to commence in Q4`09, all variants will be identified and characterized using a methodology summarized in the figure below.



In addition, the SAEC is exploring a collaboration with Illumina to sequence 40 coamoxiclav livery injury cases at high coverage. The working hypothesis underlying this research is liver injury results from a combination of common HLA risk alleles and a small number of low frequency variants — or variants in a small number of genes — with sufficiently large effects to be identified in relatively small sample sizes with reference controls. The overall objective of this study will be to identify rare variants associated with drug-induced liver injury (aggregate

protein-disrupting variants and individual variants) and to assess the role of rare coding variants in DILI risk with known genetic risk factors. The exact details of this study are still under deliberation, but it will study a fairly large number of coamoxiclav cases with extreme cholestatic DILI and high drug causality (RUCAM ≥ 6). We aim to commence this study in Q1`10. The data analysis for this study will be completed by the DACC at Columbia University, in conjunction with Duke University.

SAEC PHASE 2 PROJECT SUMMARY

A. Scientific Implications of the SAEC's Phase One Results

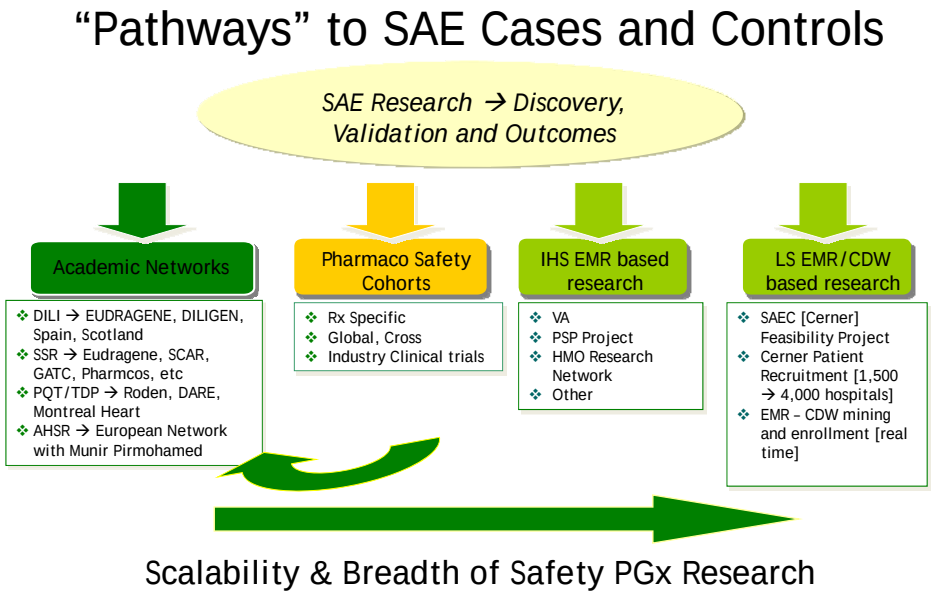
A strong collaborative base is necessary for success
It is clear from the SAEC's phase one SSR and DILI GWAS studies, the available genotyping and sequencing technologies in combination with the scope of the SAEC's collaborative networks, can enable effective genomic research on drug-induced SAEs. The SAEC phase one accomplishments were generated quickly and effectively, with modest resources. Academic collaborators and healthcare providers are keen to benefit from the collaborative capabilities the SAEC has established to support SAE translational research. The SAEC has established a global orientation to deal with ethnic diversity, it is driving efforts to standardized phenotype definitions for several SAE/ADRs of greatest interest to the Consortium to enable better and more efficient case ascertainment via electronic medical records, and it has established a genotyping core and data analysis and coordinating capabilities essential to SAE research.

Important DNA variants can be identified from a small number of cases
From a scientific perspective, the SAEC phase one results have proven that robust statistical inferences are possible from relatively small SAE cohorts (e.g. our DILI research results, generated with 50-150 well characterized cases) and larger numbers of well-matched controls.

More cases will be required to fully explore drug and ethnicity-specific effects
The more challenging finding of phase one is that although there will likely be important genetic effects influencing many SAEs, many of these effects will be drug and ethnicity-specific, and vary in their predictability and clinical utility. SAE clinical cohorts, in the future, will need to be designed to recruit adequate numbers (>30) of specified drugs or drug classes to permit well-powered studies on a genome-wide scale.

In phase one, the SAEC relied heavily on academic collaborations (e.g. Diligen and EUDRAGENE) to source appropriate cases. The majority (61%) of the SAEC's DILI cases were recruited prior to the formation of the SAEC. The SAEC was very effective in leveraging previous recruitment efforts and minimizing *de novo* recruitment. Collaborative partnerships with academic SAE research networks will be essential to continue in phase two. However, alone they do not possess the size and scale required for SAEC to recruit drug-specific cases across a broader ethnic base. Consequently, the SAEC has/is exploring the feasibility of working with electronic medical record (EMR) providers (e.g. Cerner) and health care providers

(e.g HMORN) to identify and obtain greater numbers of cases across different SAE-causing drugs. The rarity of such SAEs and the absence of effective government surveillance/research networks make it extremely difficult for any one company/research entity to accrue enough SAE cases to meet our requirements in phase two. As illustrated below, the SAEC will need to combine the capabilities of academic network, member pharmaceutical companies, integrated health care providers, and EMR companies to meet our recruitment objectives. The SAEC will be piloting the use of 1) EMR-based identification and 2) web-based case enrollment to accomplish its phase two scientific objectives.



Immune-mediated adverse events may be most tractable

One of the most significant scientific findings from SAEC's first phase is that some HLA risk alleles confer SAE/ADR risk for multiple drugs. *HLA-B*5701* was discovered as a major risk factor of DILI due to flucloxacillin. It is already well documented that this allele also confers a high risk of hypersensitivity reaction to abacavir. It is likely that *HLA B*5701* and other such HLA risk alleles are involved across multiple drugs, where the involvement of the immune system is paramount. (e.g. serious skin rashes, acute hypersensitivity reaction, or DILI). As summarized in the table below, incorporating SAEC and external findings, *HLA-B*5701* and *UGT1A1*28* both confer genetic risks associated with different drugs and different adverse reactions.

Genetic Influence on ADR Risk

Selected Examples

Drug	Adverse Drug Reaction		Genetic Risk Factor		
	Reaction	Prevalence	Risk Allele	Freq.[1]	Effect[2]
Clopidogrel	Cardiovascular events	0.13	*CYP2C19*2/3/4/5*	0.03	3
Gefitinib	Diarrhea	0.28	*ABCG2* Q141K	0.07	5
Isoniazid	Hepatotoxicity	0.15	*CYP2E1*1 & NAT2*	0.13[3]	7
Co-amoxiclav	Hepatotoxicity	<0.001	*HLA-DRB1*1501*	0.20	10
Irinotecan	Neutropenia	0.20	*UGT1A1*28*	0.32	28
Ticlopidine	Hepatotoxicity (cholestatic)	<0.001	*HLA-A*3303*	0.14	36
Tranilast	Hyperbilirubinemia	0.12	*UGT1A1*28*	0.30	48
Flucloxacillin	Hepatotoxicity	<0.001	*HLA-B*5701*	0.04	81
Allopurinol	Severe cutaneous reaction	<0.001	*HLA-B*5801*	0.15	678
Abacavir	Hypersensitivity reaction	0.08	*HLA-B*5701*	0.04	>1000
Carbamazepine	Stevens-Johnson	<0.001	*HLA-B*1502*	0.04	>1000

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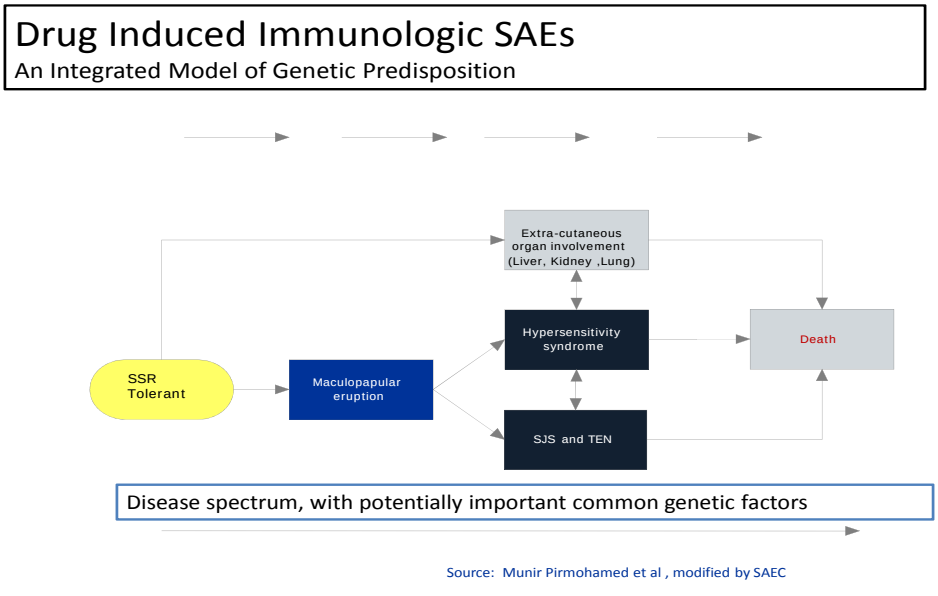
It would be of both scientific and clinical importance, if more genetic and clinical data were available across different SAE causing drugs, where the effect involves the malfunction of the immune system (i.e. serious skin rashes, acute hypersensitivity reaction, or DILI). At the July 2009 SAEC Board of Directors meeting, Dr. Munir Pirmohamed of the University of Liverpool presented the hypothesis of an integrated model of a spectrum of drug-induced immunologic reactions (e.g. serious skin rashes, acute hypersensitivity reaction, or DILI) with potentially important common genetic factors at work (see below figure). Phase 2 of the SAEC's research will focus heavily on exploring this hypothesis through a focus on expansion of our current SSR and DILI cohorts, while initiating an international acute hypersensitivity reaction network.



Source: Munir Pirmohamed et al , modified by SAEC

B. <u>SAEC Phase 2 -- Key Objectives</u>

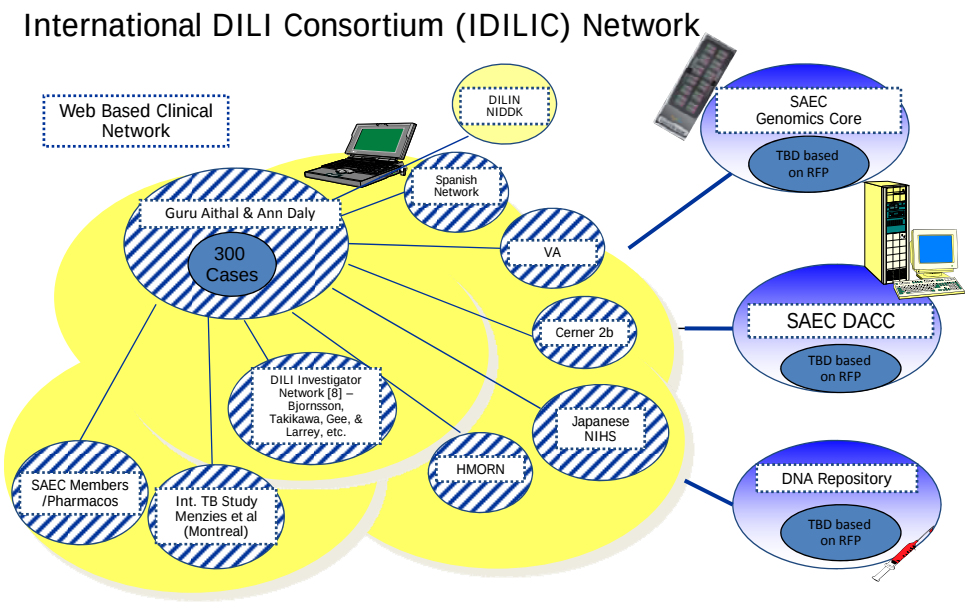
Phase 2 of the SAEC's research will explore this hypothesis through focused expansion of our current SSR and DILI cohorts, while initiating an international acute hypersensitivity reaction network. The SAEC will focus on achieving a more integrated biological understanding of the genetics associated with drug induced immunologic SAEs (i.e. AHSS, SSR & DILI) by pursuing the following objectives:

1. Expand our DILI cohort with a mix of networks to better define genetic mechanisms contributing to DILI within specific drugs and drug classes.
2. Establish an AHSS network (i.e. case collection, GWAS, and analysis) to define possible genetic mechanisms. Expand our SSR cohort with a mix of networks to better define genetic mechanisms contributing to DILI within specific drugs.
3. Integrate SAEC member SAE cohorts for GWAS, analysis and inclusion where they can add breadth to this drug induced immunologic SAEs focus.
4. Complete a SAE Phenotype Standardization Project (PSP) on the target SAEs, with the Wellcome Trust and the FDA, to improve the SAECs ability to leverage EMR based recruitment to support SAE translational research.
5. Partner with large scale HMO and government healthcare systems (*with quality EMRs*) to enhance SAE case recruitment and better establish long-term SAE research channels for individual pharmaceutical safety research.
6. Complete two genome-wide genotyping studies (coamoxiclav liver injury and clozapine agranulocytosis) to investigate the role of low frequency variants in SAE risk.
7. Continue the development of optimal genotyping and sequencing approaches for SAE genetic research

C. Expand our DILI cohort with a mix of networks to better define genetic mechanisms contributing to DILI within specific drug classes

This expansion will be led by Guru Aithal at the University of Nottingham and Ann Daly at the University of Newcastle. Newcastle University will serve as the coordinating center for the International DILI Consortium (IDILIC). The recruitment target will be to recruit 300 additional DILI cases between Q1`10 and Q2`12, with a focus on aggregating DILI cases of the key drugs underrepresented in SAEC phase one study (e.g. anti-TB, antibiotics (non-coamoxiclav), anticonvulsants, NSAIDs, and immunosuppressants). In addition, it is hoped member cohorts for compounds such as ximelagatran and lumiracoxib can be integrated into the SAEC overall DILI cohort. The SAEC will continue to work on its partnership with NIDDK's DILIN, leveraging wherever possible the resources and expertise of both groups.

These cases will be recruited retrospectively via an emerging variety of clinical networks over the course of phase two. All cases will be enrolled via a SAEC-hosted IDILIC case enrollment website. This hosted website will be developed and hosted by Cerner, Inc. The figure below summarizes the proposed structure of the IDILIC.



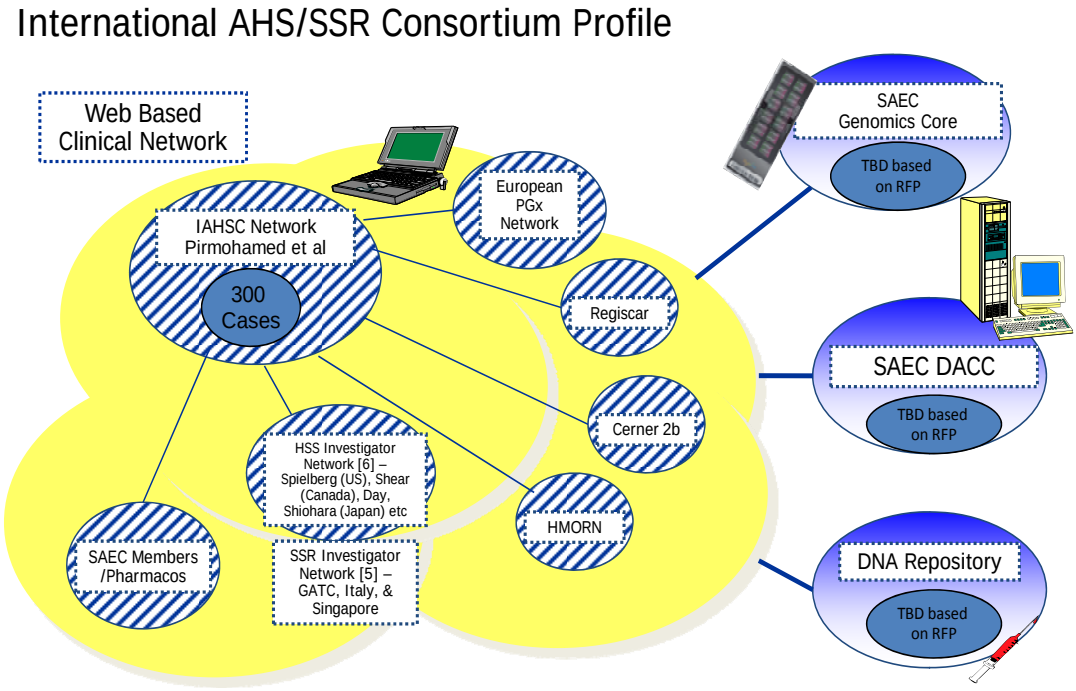
D. Establish an AHSS network (i.e. case collection, GWAS, and analysis) to define possible genetic mechanisms, and the expansion of our SSR cohort with a mix of networks to better define genetic mechanisms contributing to DILI within specific drug classes

This vital network will be led by Munir Pirmohamed at the University of Liverpool. Liverpool will serve as the coordinating center for the International AHSS/SSR Consortium (IAHSS/SSRC). The recruitment target will be to recruit 200 additional SSR cases between Q1`10 and Q2`12, with a focus on aggregating SSR cases of the key drugs underrepresented in SAEC phase one study (e.g. co-trimoxazole, lamotrigine, phenytoin, beta-lactam, carbamazepine, and allopurinol). The SAEC will continue to explore collaborative opportunities with RegiSCAR, leveraging wherever possible the resources and expertise of both groups. In addition, the SAEC will leverage existing collaborations with GATC/Vancouver, EUDRAGENE, and the Italian SSR network.

The recruitment target will be to recruit 200 new AHHS cases between Q1`10 and Q2`12, with a focus on aggregating AHSS cases on the key causal drugs (e.g. aromatic anticonvulsants, lamotrigine, sulfonamides, allopurinol, dapsone, minocycline, and nevirapine). In addition, it is hoped member cohorts for compounds such as Abacavir can be integrated into the SAEC overall AHSS cohort. This research will also explore, to the extent possible, "route of administration" (i.e. topical, IM & IV intramuscular). The working hypothesis being the IV route appears more likely to cause AHSS. Ethnic factors will also be explored. (e.g. effects are caused by the efficiency of antigen presentation in the skin).

The AHSS and SSR cases will be recruited retrospectively via an emerging variety of clinical networks over the course of phase two. All cases will be enrolled via a SAEC hosted

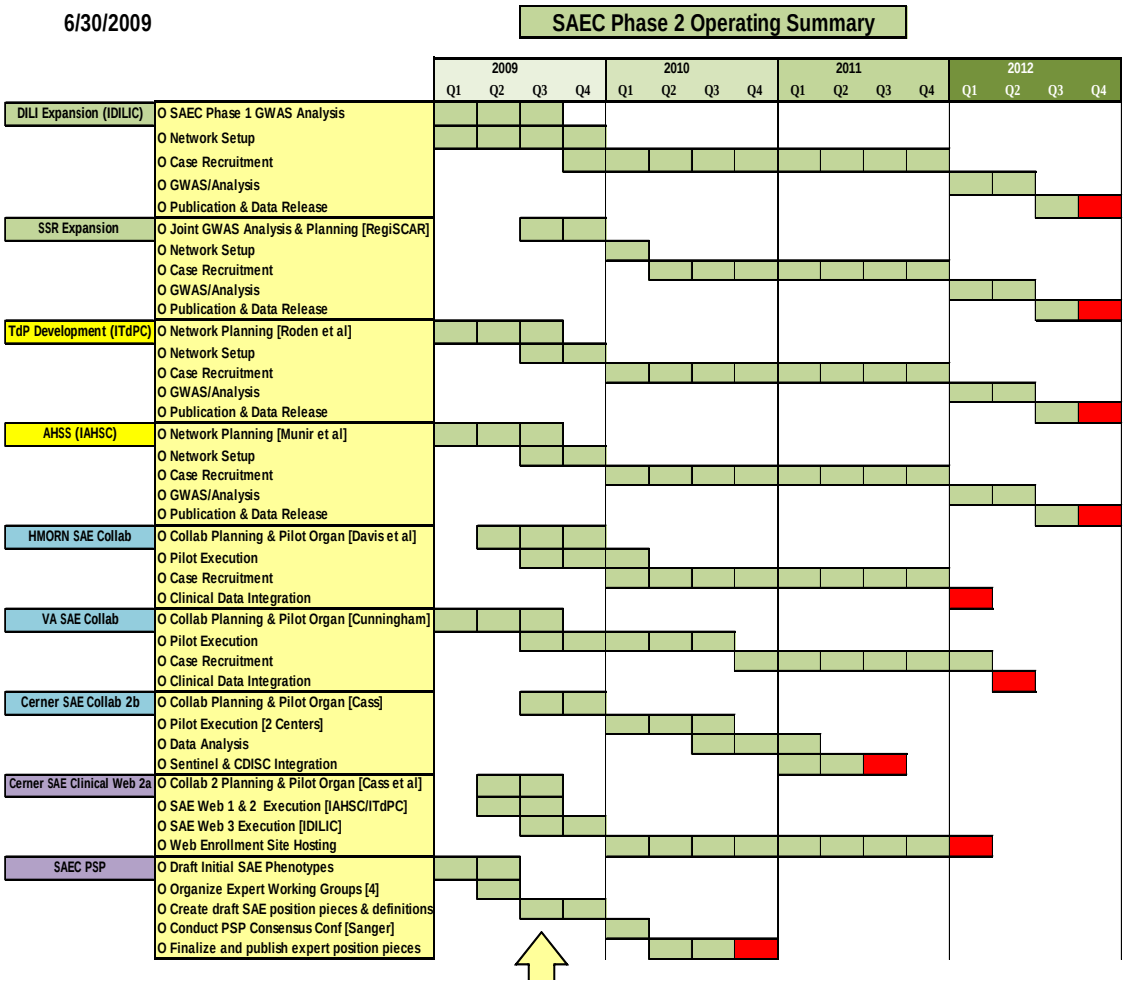
IAHSS/SSRC case enrollment website. This hosted website will be developed and hosted by Cerner, Inc. The figure below summarizes the proposed structure of this consortium.



Fundamental to the SAEC's phase two activity, is a scaling up of its collaborative research with large scale research networks, specifically the HMO Research Network and the VA healthcare system. Building on feasibility efforts initiated in phase one (*using EMRs and large clinical data warehouses*), the SAEC will move to put in place research networks/capabilities which will facilitate both entities enrolling patients into the above mentioned SAEC sponsored international AHSS, SSR, and DILI clinical consortia.

Summarized below is the aggregate research operating plan and associated milestones, as of October 2010.

SAEC Phase 2 Operating Milestone Summary



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