Purification of SARS Hyperimmune Globulins

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Severe Acute Respiratory Syndrome

Global Outbreak 2002-2003

Countries	Cases	Death	Fatality
China	5,327	349	7%
Hong Kong	1,755	299	17%
Taiwan	346	37	11%
Canada	251	43	17%
Singapore	238	33	14%
Viet Nam	63	5	8%
All countries	8,096	774	9.6%

From Nov 16, 2002 to Jul 13, 2003. Source: WHO

Current First-line Treatment for SARS

- Ribavirin and Steroids

  Retrospective study of 323 patients (Princess Margaret & Wong Tai Sin Hospitals, Hong Kong)

  64%, rapid disease progression
  21%, intensive care
  13%, ventilator support
  8%, death

  Tsui et al *Emerg Infect Dis* 2003; 9: 1064-9

Convalescent Plasma Treatment of SARS

- Zhou *et al*, *Zhonghua Yi Xue Za Zhi*. 2003; 83: 1018-22

  a 74-year-old Beijing patient received 50 ml

  Outcome: complete recovery

- Soo *et al*, *Clin Microbiol Infect*. 2004; 10: 676-8

  28 Hong Kong patients received 200-400 ml

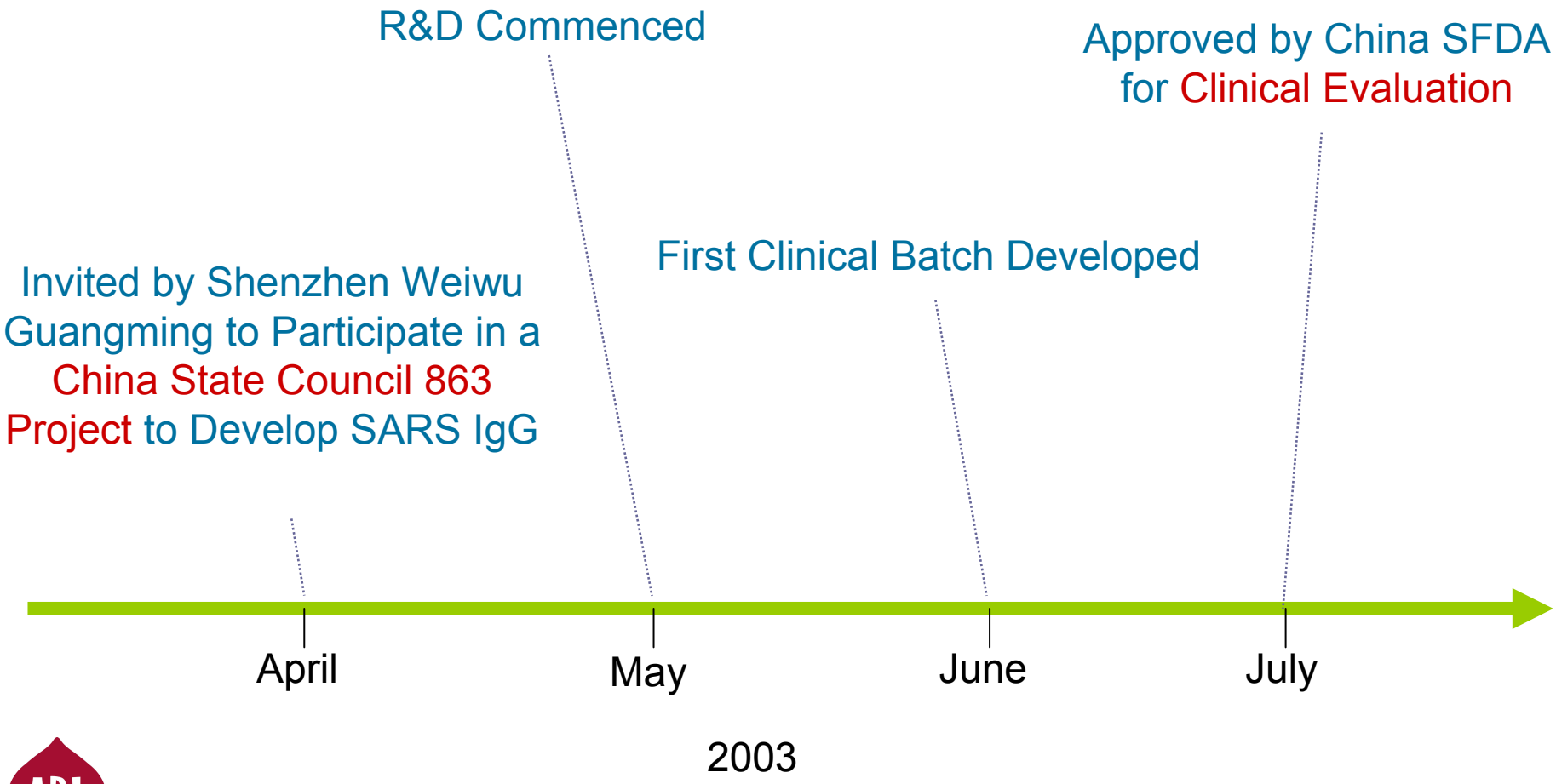
  Outcome: no death

  shorter disease course

  good responses before day-16

World's First SARS Investigational Drug

Human Hyperimmune Globulins



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Collection of Convalescent Plasma

- At least 28 days after hospital discharge
- Plasmapheresis collection of 200-400 ml/unit, (total 47 units), frozen immediately below -20 °C
- Routine screening negative for HBsAg, HCV antibody, HIV antibody and syphilis, and normal for ALT
- Tested positive for anti-SARS antibodies

Determination of anti-SARS Titer

- ELISA (commercial kit)
- Immuno-Fluorescence Assay (supported by the Chinese University of Hong Kong)

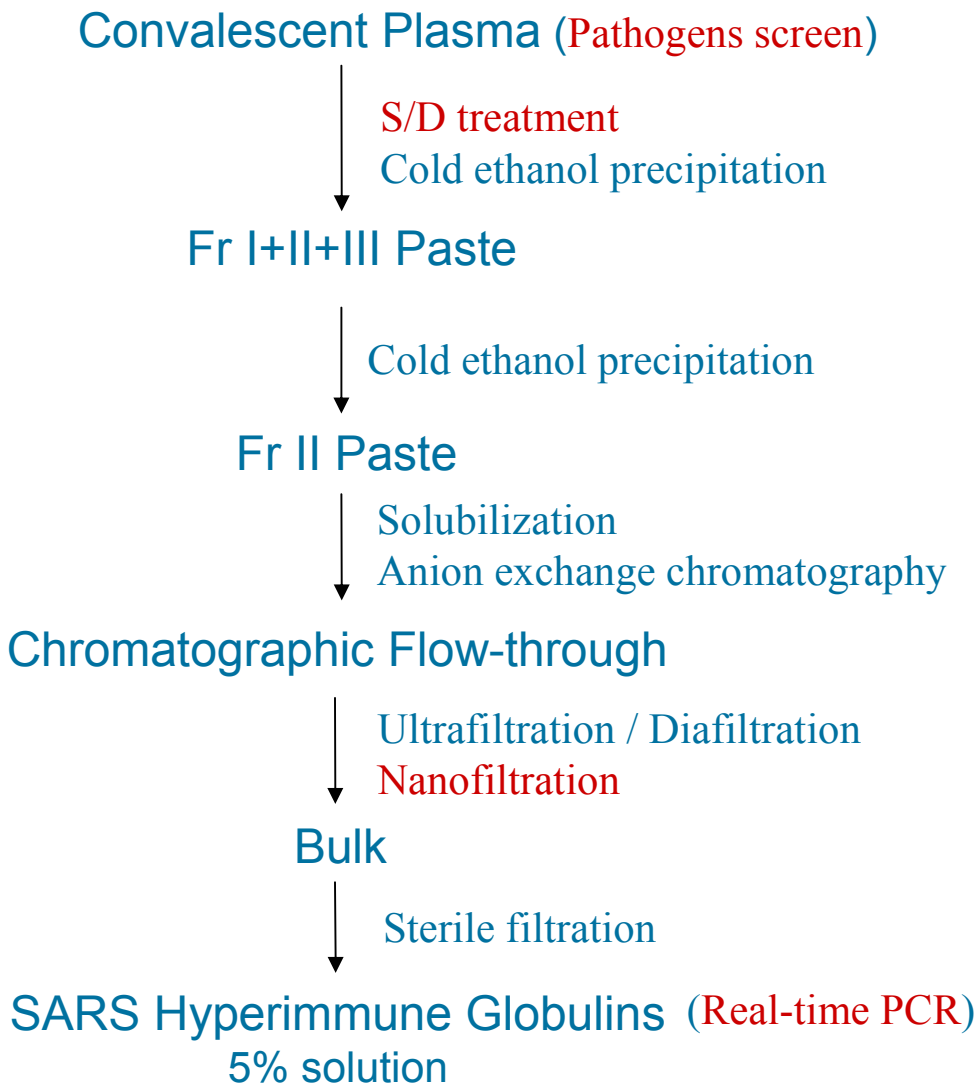
- Neutralizing Antibody Test (provided by the Chinese National Institute for the Control of Pharmaceutical and Biological Products)

Anti-SARS Titer of Convalescent Plasma

ELISA Titer	Number of Plasma Units
1 : 2	2
1 : 4	5
1 : 8	12
1 : 16	17
1 : 24	6
1 : 32	3
1 : 48	2

ELISA titer of pooled convalescent plasma, 1 : 12

Process: Human Hyperimmune Globulins



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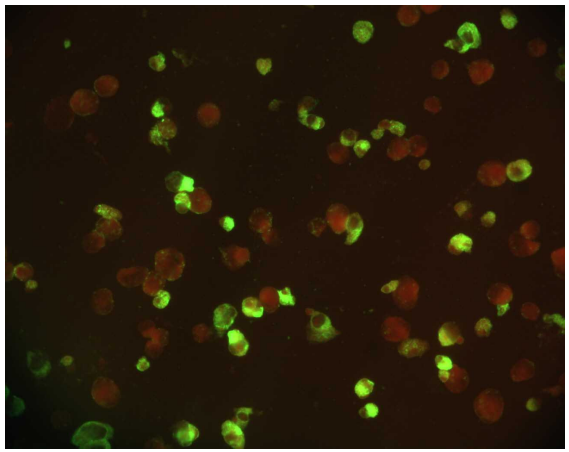
Titer of Human Hyperimmune Globulins

ELISA Titer	1 : 83
IFA Titer	1 : 1,600
Neutralizing Titer	1 : 200



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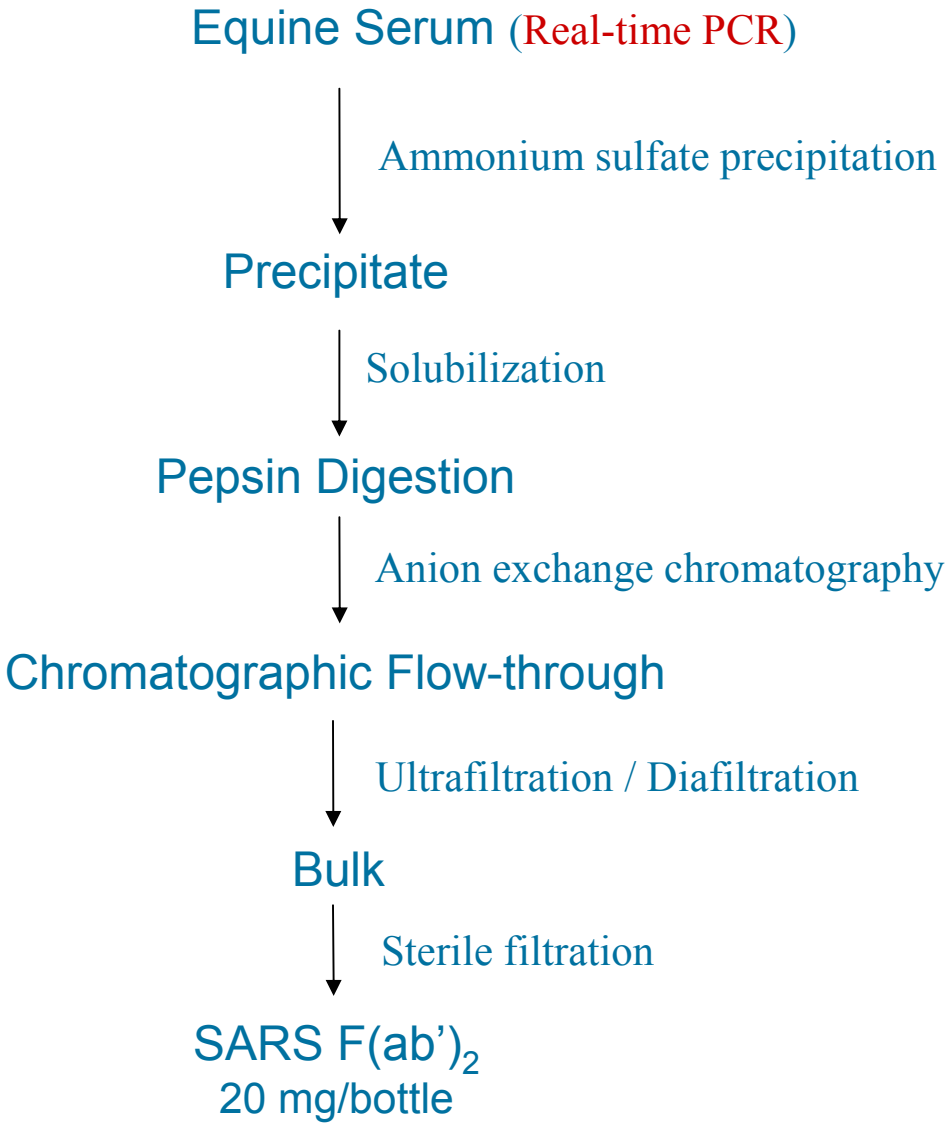
PCR Detection of SARS Virus RNA

- RNA extracted from the human hyperimmune globulins
- 200 ng RNA used in the quantitative real-time PCR reactions, with proper negative, positive and spiking controls, following *Biochem Biophys Res Commun*. 2003; 312: 1290-6
- Result: Negative for SARS virus RNA

Characteristics of Human Hyperimmune Globulins

Assay items	IVIG specifications	SARS IgG
pH	3.8 – 4.4	4.1
Purity	≥ 95%	99%
Mono + Dimer	≥ 95%	100%
ACA	≤ 50%	11%
PKA (IU/ml)	≤ 35	Not detected
TnBP residue	≤ 10 ppm	< 2 ppm
Triton residue	≤ 10 ppm	< 5 ppm

Process for Equine F(ab')$_2$ Fragments



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Equine Serum with SARS Antibodies

- SARS virus inactivated with formaldehyde, inactivation confirmed in cell culture
- Immunization/re-immunization of horses
- Much higher neutralizing titer 1:10,280, compared with human convalescent plasma
- Negative for SARS virus RNA, with the quantitative real-time PCR

Characteristics of SARS F(ab')$_2$

Assay items	Antitoxin specifications	SARS F(ab')$_2$
Protein conc.	≤ 17%	2.1%
F(ab')$_2$ Purity	≥ 60%	64.3%
pH	6.0 – 7.0	6.59

Comparison: Human v.s. Equine

Human SARS Globulins	Equine SARS $F(ab')_2$
• Very limited	• Unlimited
• Lower titer	• Much higher titer
• Less antigenic	• More antigenic

Conclusion

- Purified and concentrated SARS hyperimmune globulins are prepared and ready to be further evaluated.

- The SARS hyperimmune globulins may help to better contain future SARS outbreaks

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