



Early Development of the Icon Molecule for Gene Therapy of Cancer and Macular Degeneration

Alan Garen*

Department Molecular Biophysics and Biochemistry, Yale University, USA

*Corresponding author: Alan Garen, Department Molecular Biophysics and Biochemistry, Yale University, USA.

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Mini-Review

I conceived the Icon strategy for cancer immunotherapy in 1996 after reading a paper showing that Tissue Factor (TF) is expressed on the surface of vascular endothelial cells and tumor cells in invasive breast cancer but not in benign breast cancer [1]. Although it was not tested in this paper, I assumed TF also is not expressed on the surface of vascular endothelial cells in normal tissues and it might provide a specific marker for the tumor vasculature. TF is a cell-surface transmembrane receptor containing an extracellular domain that binds factor VII (fVII) with high affinity and specificity, initiating the blood coagulation pathway [2] (Figure 1). TF exists as an integral membrane protein in the intact wall of normal blood

vessels, preventing contact with fVII circulating in the blood, in contrast to tumor blood vessels in which the vessel wall is damaged and leaky [3], exposing TF to fVII. Thus, TF could serve as a specific molecular target and fVII as a specific targeting molecule for the pathological neovasculature (PNV). Accordingly, I designed the Icon as a heavy-chain VH immunoconjugate antibody [4] composed of factor VII as the targeting domain attached either to a mouse or human Fc domain (Figure 2), similar to a Camelid antibody composed of heavy-chain dimers devoid of light chains [5]. A mutation was introduced into the active site of fVII to inhibit the initiation of the blood coagulation pathway, which might result in pathological blood clots [6].

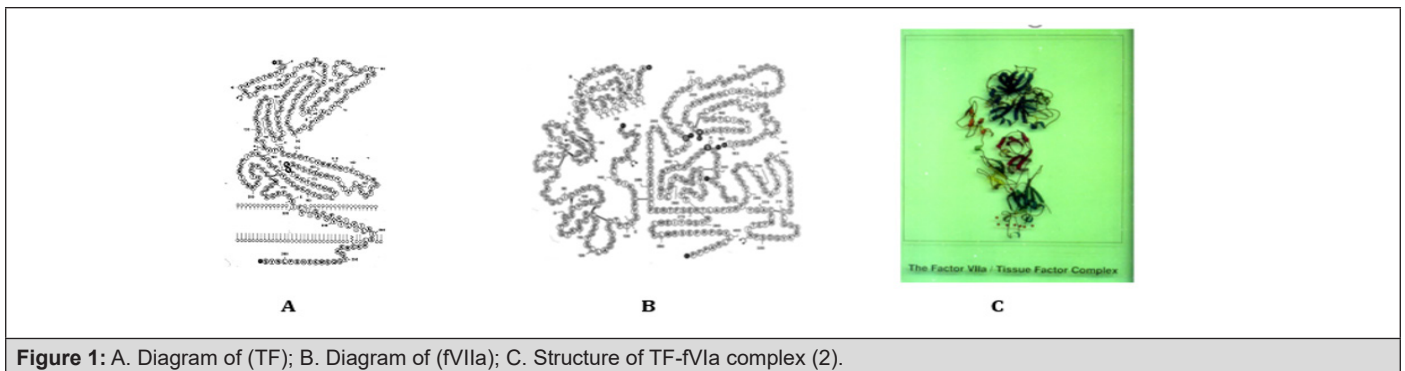


Figure 1: A. Diagram of (TF); B. Diagram of (fVIIa); C. Structure of TF-fVIIa complex (2).

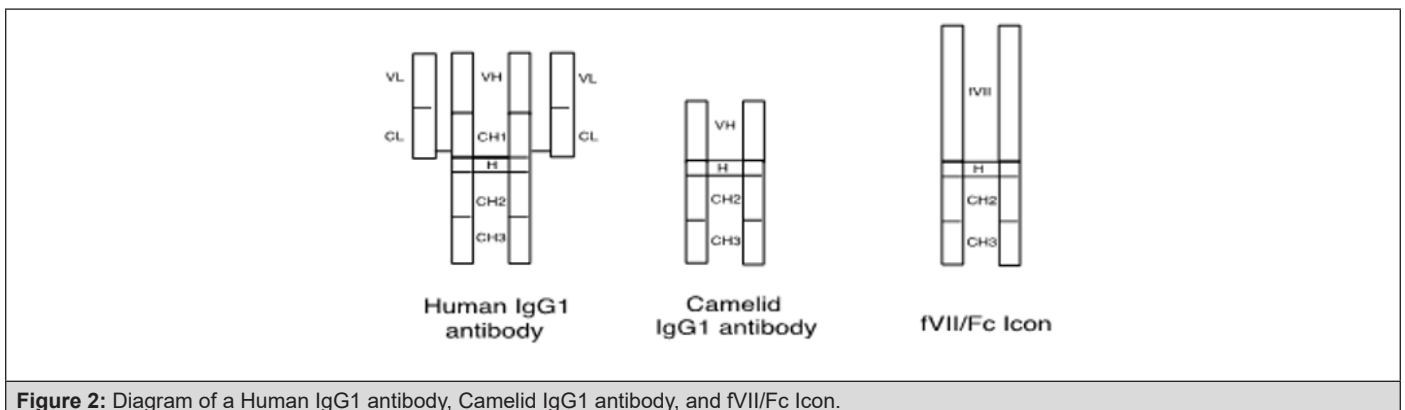


Figure 2: Diagram of a Human IgG1 antibody, Camelid IgG1 antibody, and fVII/Fc Icon.

In 1997 Zhiwei Hu joined my laboratory after receiving his doctorate from Hunan Medical University, and together with Ying Sun we demonstrated the efficacy and safety of the Icon in eradicating a human tumor xenograft in a mouse gene therapy model [7-9]. The Icon cDNA was incorporated into a replication-defective adenovirus vector (AV) that was administered either by intratumor or intravenous injections. The AV-infected cells synthesized and secreted the Icon protein into the blood stream, resulting in binding of the Icon to TF on the tumor PNV and tumor cells, causing an immune attack by natural killer cells that eradicated the tumor PNV and tumor cells without evidence of damage to normal tissues. Of special significance was an experiment using a mouse model of metastasis containing two separate human tumor xenografts: one xenograft was injected with the Icon adenovirus vector, resulting in a cytolytic immune attack not only against the injected tumor xenograft but also against the distant noninjected tumor xenograft [9]. Yale University applied for a patent of the Icon on June 14, 2000 (US14216199P), which was granted on August 2, 2005 (US6924359B1), and the Icon technology was licensed to a start-up company called Iconics Therapeutics (IT). Our next Icon project concerned the exudative or wet form of macular degeneration (wMD), a major cause of vision impairment and blindness associated with aging [10]. wMD involves the PNV in the choroidal tissue located behind the retina, which invades and damages the macular region of the retina. As we had shown that the Icon binds to TF specifically in the PNV of solid tumors causing eradication the PNV, I considered that the Icon might have a similar effect on the choroidal PNV. In 2001 I contacted Dr. Henry Kaplan in the Department of Ophthalmology and Visual Sciences, University of Louisville, proposing a collaboration to test the Icon in his mouse model of laser-induced wMD. Our results showed that intraocular or intravenous injection of Icon protein or an adenoviral vector carrying the Icon cDNA caused eradication of the choroidal PNV without evidence of collateral damage to the normal choroidal and retinal vasculature [11, 12]. Similar results were obtained in a pig model of wMD [13]. Experiments on photodynamic therapy with the Icon also were initiated in my laboratory and later continued by Zhiwei Hu [13].

Although most of our experiments of the Icon for treatment of solid tumors and wMD involved gene therapy using an adenovirus vector to deliver the Icon cDNA, gene therapy trials were put on hold following the tragic death of Jessie Gelsinger soon after receiving on September 13, 1999 an intravenous injection of an exceptionally high dose of a replication-deficient adenoviral vector carrying the normal OTC gene to treat his partial ornithine transcarbamylase (OTC) deficiency [14]. This procedure resulted in a massive immune response to the adenoviral vector causing formation of blood clots. Instead of using a gene therapy protocol, IT decided to produce the Icon protein *ex vivo* for injection *in vivo*. However, having failed to produce sufficient active Icon protein for treating cancer, IT has focused on treating wMD by intraocular injections of the Icon

protein, which should be more efficacious for treating wMD than current protocols involving intraocular injections of antibodies against VEGF or VEGF receptors [15]. VEGF antibodies inhibit the development of the choroidal PNV and require continuing injections, in contrast to the Icon that eradicates the choroidal PNV [16]. Clinical trials of the Icon for wMD organized by IT are in progress [17]. To continue developing the Icon gene therapy approach for treating solid tumors, I proposed in 2000 a collaboration with Dr. Albert Deisseroth, who was then Chief of Medical Oncology at Yale University School of Medicine, to work with Zhiwei Hu and me on arranging a clinical trial of the Icon for treating melanoma by intratumor injections of a replication-incompetent adenovirus vector carrying the Icon cDNA. After Deisseroth left Yale in 2002 to become President of the Sidney Kimmel Cancer Center (SKCC) in La Jolla, California, he and I were Principal Investigators on a Program Project Grant to the SKCC to begin this project. We arranged with Molecular Medicine Biosciences (now known as SFAC Pharma in San Diego) to produce the Icon adenovirus vector under GMP conditions, but the final production could not be used clinically because of contamination with replication-competent recombinant adenoviruses, and the project could not be completed. The key advantages of the Icon gene therapy protocol for treating solid tumors, notably that the Icon appears to be efficacious for treating all types of solid tumors by eradicating the PNV and associated tumor without collateral damage to the normal vasculature, encourages further testing of other vectors [18, 19] that might avoid the initial problems associated with an adenovirus vector.

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