

Antibodies Targeting K_V Potassium Channels: A Promising Treatment for Cancer

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Abstract

Voltage-gated potassium channels are transmembrane proteins that allow flow of potassium across the membrane to regulate ion homeostasis, cell proliferation, migration, cell volume, and specific processes such as muscular contraction. Aberrant function or expression of potassium channels can underlie pathologies ranging from heart arrhythmia to cancer; the expression of potassium channels is altered in many types of cancer and that alteration correlates with malignancy and poor prognosis. Targeting potassium channels therefore constitutes a promising approach for cancer therapy. In this review, we discuss strategies to target a particular family of potassium channels, the voltage-gated potassium channels (K_V) where a reasonable structural understanding is available. We also discuss the possible obstacles and advantages of such a strategy.

Keywords: potassium channels, immunotherapy, antibodies, cancer

Introduction

DURING TRANSFORMATION, cancer cells acquire characteristics that provide improved survival rates and better defense against different hostile environments and death stimuli.¹ For example, overexpression of oncogenes and the inhibition of tumor suppressor proteins allow aberrant regulation in the signal pathways that orchestrate cellular processes, such as proliferation, metabolism, cell death resistance, migration, and invasion. For decades, the main strategy for cancer treatment has been to defeat the fast proliferation of tumor cells, that is, to target only one of these aberrant processes.

Currently, better understanding of cell signaling regulation in cancer is leading to a paradigm shift, with new tools and new therapeutic targets, allowing researchers to design new strategies to treat cancer in a safer and more efficacious way. In recent years, it has become increasingly clear that many ion channels are aberrantly expressed in cancer cells and that they are crucial in the regulation of transformation, migration, proliferation, and invasion.^{2–6} Because ion channels are typically plasma membrane proteins, functional regions are accessible from the outside; thus, there is growing interest in designing a therapy using antibodies that block activity. Treatments that block expression are also becoming a focus in cancer treatment research. Here, we summarize recent findings, focusing on a particular family, the voltage-gated potassium channels (K_V).

Potassium Channel Function and Regulation

K_V channels allow transport of potassium across the plasma membrane in response to the electrochemical gradient.⁴ They

are transmembrane proteins that belong to the structurally related voltage-gated ion channel superfamily.⁷ This group encompasses the products of 40 different genes and is divided into 12 subfamilies. Four monomers surround the permeation pathway, with each monomer having six transmembrane segments (termed S1–S6) and a pore loop between the S5 and S6. Both N- and C-termini are intracellular (with one exception, $K_{Ca}1.1$, which has an extracellular amino terminus and an additional transmembrane segment, S0); therefore, only three loops (S1–S2, S3–S4, and part of S5–S6) protrude into the extracellular milieu (frequently termed E1, E2, and E3).

For a long time, K_V channels were studied almost exclusively from the point of view of their functions as modulators of excitability and controllers of membrane potential in excitable cells of the nervous and muscular systems. In these cells, potassium efflux causes hyperpolarization that counteracts the depolarization induced by sodium or calcium channels. Nevertheless, it is now generally acknowledged that K_V channels are fundamental for many other natural cell processes, either dependent or independent of cellular ionic homeostasis. Events as crucial as cell proliferation, control of cell volume, or cell adhesion depend on the function of K_V channels.^{8–12}

As anticipated, due to the canonical roles of potassium channels, most identified mutations in K_V channels lead to altered excitability of neurons and muscle cells. Loss-of-function mutations can cause hyperexcitability leading to epilepsy or to cardiac arrhythmias such as long QT syndrome (LQT; e.g., $K_V7.1$ ¹³). Mutations in $K_V7.2$ or $K_V7.3$ channels are correlated with neonatal epilepsy and benign familial

neonatal convulsions,^{14,15} whereas K_v7.4 is correlated with deafness¹⁶ (autosomal dominant 2a type). Aberrant function of members of the K_v1 family, in particular K_v1.3 and K_v1.5, correlates with central neural system disorders,¹⁷ atrial fibrillation,¹⁸ and immune abnormalities^{19–21}; whereas loss of function or mutations in K_v1.1 and K_v1.2 induce convulsions, ataxia episodes, and myokymia disorders,^{22,23} and mutations in K_v11.1 cause Type 2 LQT.^{24,25} The exquisite equilibrium between ion channels required for proper function of excitable cells is also reflected in the pathological effects of gain-of-function mutations. For example, such mutations in K_v7.1 cause familial atrial fibrillation, short QT syndrome, or type 2 diabetes mellitus.²⁶ Similarly, excessive activity of K_v10.1 underlies developmental defects that occur with seizures and cognitive impairment, such as Temple–Baraitser and Zimmermann–Laband syndromes^{27–31}; interestingly, excess of function of other potassium channels (SK3, K_{Ca}2.3) has been identified in other patients with Zimmermann–Laband syndrome.³²

In some cases, a loss of ion selectivity as a result of a mutation can be the basis for pathological phenotypes. Although not reported for K_v channels, this mechanism has been described for K_{ir}3.4. It has been shown that a mutation in the structure of this channel results in loss of selectivity for K⁺ and a higher permeability for Na⁺, inducing severe aldosteronism³³; nevertheless, the intimate molecular mechanism is still unclear.

Potassium Channels in Cancer

During growth and transformation, cancer cells survive in hostile environments and are able to invade surrounding tissue and to migrate from one tissue to another. These changes can be the result of an atypical phenotype caused by genetic and/or aberrant protein expression. Understanding how cancer cells work, the regulation of cellular processes involved in tumor development, and which proteins are involved in these processes, could help us to design new therapies with high affinity for tumor cells.

Altered potassium channel expression leads to modifications that can help the development and growth of virtually every tumor type, even those as apparently unrelated as medulloblastoma, gliomas, adrenal or breast cancer, and their blockage can therefore reduce tumor growth. The specific mechanisms leading to such a role in tumorigenesis are still under investigation and can vary from their role in volume regulation during mitosis³⁴ to interactions with cell adhesion molecules and their signaling pathways.³⁵ In most cases, it is not completely understood how cellular potassium channels undergo remodeling during proliferation, nutrient starvation, angiogenesis, migration, or invasion. The reader can find detailed recent reviews on these aspects elsewhere.⁶

Even for the case of those K_v channels where a therapeutic approach has explicitly been proposed, the mechanistic knowledge is still incomplete. It has been shown that the expression of two members of the K_v1 family, K_v1.3 and K_v1.5, channels activates proliferation, migration, and invasion^{36–38}; both K_v channels regulate crucial functions in leukocytes, such as cell proliferation, activation, migration, and apoptosis. K_v1.3 is expressed in solid tumors such as breast, colon, lung, glioma, muscle, and prostate cancers³⁹ and is downregulated in some types of leukemia.

Nevertheless, its correlation with malignancy is still not clear. K_v1.3 expression levels change during cancer development; in particular, it is expressed in early stages and downregulated in high-grade cases of prostate cancer.⁴⁰ In contrast, its expression is increased in higher stage breast cancer compared with low-grade tumors.⁴¹ In fact, the available information about K_v channels in cancer development suggests that this is a common phenomenon, and their correlation with malignancy and poor prognosis depends on the stage of the disease and the type of tissue in most cases.

Until now, the role that K_v1.3 channel plays in cancer development is mechanistically obscure. In some cases, K_v1.3 is related to proliferation and cell death, but for some others, it is related to migration and adhesion. It is important to mention that K_v1.3 is also expressed on the outer mitochondria membrane, where it regulates apoptosis, and its function in cancer could therefore be more related to cell survival.⁴² K_v1.5 is expressed frequently in medulloblastoma^{43–45} and is correlated with aggressiveness for non-Hodgkin's lymphoma.⁴⁶

Members of the K_v3 family are also involved in cancer development. K_v3.4 and K_v3.1 are sensitive to hypoxia, a common condition in solid tumors; they are highly expressed in precancerous lesions and in oral squamous cell carcinoma compared with normal oral mucosa; K_v3.4 is upregulated in other head and neck cancers as well.^{40,47,48} It has been shown that K_v3.4 and K_v3.1 are involved in metastasis and invasion processes in A549 and MDA-MB-231 cells; an increased expression of K_v3.4 and K_v3.1 correlates with proliferation, metastasis, and invasion. The expression of both K_v channels is regulated in a cell density-dependent manner, and the pattern is similar to those of hypoxia-inducible factor-1 (HIF-1) and reactive oxygen species.⁴⁹ K_v3.4 expression is also implicated in the survival strategies of irradiated leukemia cells.⁵⁰

Another potassium channel related to oxygen homeostasis is K_v11.1; in this case, by the activation of PI3K/Akt pathway, which induces HIF and vascular endothelial growth factor to promote gastric tumor progression.⁵¹ It is overexpressed in human primary cancers such as leukemia, esophageal, endometrial, pancreatic, colorectal, ovarian, and brain cancers^{52–54}; it is also expressed in precancerous lesions of the esophagus, and it has a correlation with malignant progression for adenocarcinoma. Blockage of K_v11.1 results in the inhibition of cell growth, angiogenesis, and metastasis.^{51,55} Nevertheless, K_v11.1 has recently been correlated with glucose transporter 1 (GLUT-1), but in this case, its absence represents a negative prognostic factor for colorectal adenocarcinoma. The combination of K_v11.1 and GLUT-1 deregulation can be used to identify the prostate cancer stage.⁵⁶

Abnormal expression of K_v10.1 is particularly frequent, being found in >70% of all types of cancer, and it can facilitate transformation and favor tumor progression.^{57–70} K_v10.1 regulates proliferation processes in cancer cells,⁷¹ and due to its very restricted expression pattern (K_v10.1 is only detected in neural tissue and cancer cells), it may represent a promising oncological target.^{72,73} K_v10.1 stimulates vascularization through HIF activation,^{74,75} but it also promotes migration,⁷⁶ is related to epithelial to mesenchymal transition,⁷⁷ and is required for proper primary cilium disassembly and cell cycle progression after G2.⁷⁸

Other potassium channels related to cancer are K_v9.3 and K_v2.1, correlated with cell cycle regulation, and K_v4.1,

related to proliferation. However, the mechanisms through which K_V channels are regulating all these processes are still unclear.^{79,80}

Antibodies Targeting K_V Channels for Cancer Treatment

Based on their functional profile, K_V channels constitute a very attractive target for cancer therapy: accessible, differentially expressed in cancer cells, and functionally related to pathogenesis. Nevertheless, exploiting these features is challenging. Although a large number of natural K_V channel modulators are known, lack of selectivity is a major concern with the vast majority. The modulators usually belong to one of three major categories: metal ions, organic small molecules, and venom-derived peptides. Peptides and peptidomimetics are important candidates as potent and specific inhibitors,⁸¹ but lack of selectivity limits the applicability of the first two groups.

An alternative approach to circumvent this problem would be to take advantage of the exquisite selectivity of antibodies, which have been applied successfully to other target classes; in fact, there is a growing tendency toward investigation and application of antibodies to treat neurological diseases and cancer. Besides their high affinity and specificity, antibodies offer pharmacokinetic features that can be advantageous in a therapeutic setting. Avidity for the antigen results in the antibody being concentrated where the targets are found, and the plasma half-life is also high. Moreover, their activity is not limited to the particular effect of binding to the antigen since they can elicit immunological responses against the target cells. Furthermore, antibodies can be engineered to reduce antigenicity, manipulate biodistribution, and improve their immunogenic properties.⁸²

Nevertheless, to date, only one ion channel antibody-based therapy has reached the level of clinical trials; this is a polyclonal sheep antibody against a nonfunctional form of P2X7, which is being evaluated for topical treatment of basal cell carcinoma.^{81,83}

The selectivity and specificity of antibodies against K_V channels can be also used for diagnostic and prognostic purposes, but we will limit our description to therapeutic strategies in cancer. The therapeutic potential of antibodies against ion channels in general has recently been exhaustively reviewed elsewhere.^{84,85} Of note, antibodies can modulate ion channels without directly inhibiting their function, for example, by inducing channel internalization as it is the case for KCNK9⁸⁶ or the calcium entry channel Orai1.⁸⁷ Here, we focus on examples where inhibitory antibodies against K_V channels relevant for cancer pathology have been generated, and we discuss possible causes and solutions for the low success rate in generating functional antibodies.

The simplest and most widely used approach for antibody generation, using peptide immunization, has shown limited success with ion channels. It seemed straightforward to obtain polyclonal antibodies able to inhibit K_V channels. For example, $K_V1.3$ -BA and $K_V3.1$ -BA, rabbit polyclonal antibodies with high affinity, showed ability to reduce whole-cell current in HEK-293 and NG108-15 cells.⁸⁸ $K_V3.1$ has been correlated with important cellular processes such as proliferation and migration⁴⁹ in oligodendrocyte progenitor cells in the nervous system. The use of antibodies derived from rabbit specific for $K_V3.1$ inhibits proliferation and migration of

oligodendrocytes.⁸⁹ However, the limited availability and the limited suitability for modifications of rabbit polyclonal antibodies preclude seeking evidence that these agents have the desired effect in cancer cells. Similarly, monoclonal antibodies that specifically recognize individual K_V channels are commonly available, but monoclonal functional antibodies are more elusive.

The selection of the antigen to obtain monoclonal antibodies able to inhibit ion channels is one of the major hurdles in the process. Several factors contribute to this difficulty, among others the high degree of conservation between channels at the extracellular loops. Over a decade ago, our laboratory took advantage of the (at the time only inferred, but then confirmed by cryo-EM investigations^{90,91}) peculiar structure of the KCNH family of channels ($K_V10.x$, $K_V11.x$, and $K_V12.x$), which show a particularly large extracellular loop between the transmembrane segments S5 and S6 (i.e., E3), immediately beside the selectivity filter.

Because $K_V10.1$ shows a remarkably selective expression in cancer cells, and the overexpression of $K_V10.1$ has been documented in 70% of all types of human solid tumors,^{2,4} the clinical potential of targeting this channel is high. Its inhibition reduces tumor growth *in vivo*, highlighting its potential as a therapeutic target in cancer. We therefore set out to produce a monoclonal antibody able to inhibit $K_V10.1$ in intact cells while preserving $K_V10.2$ and K_V11 channels. The latter (most frequently termed HERG) poses a significant safety concern

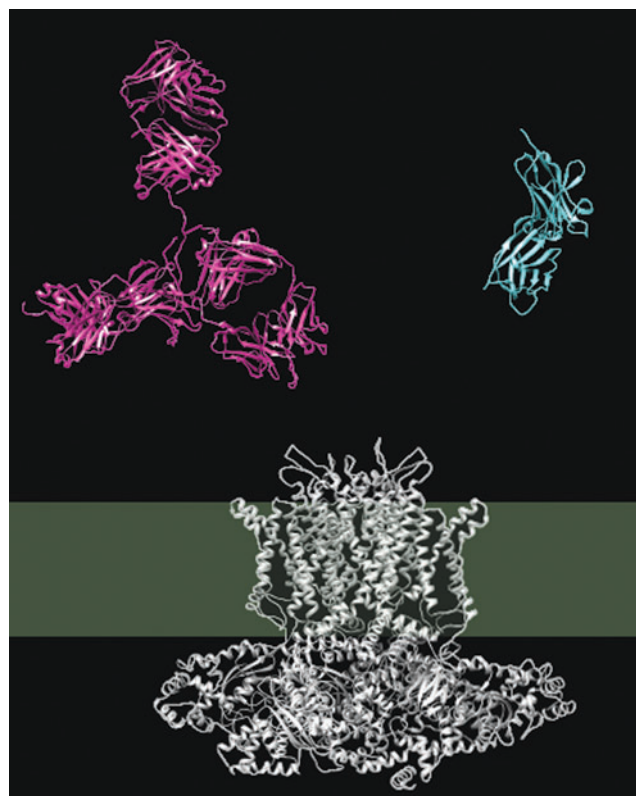


FIG. 1. Comparison of the structures of an immunoglobulin (red; IgG1, PDB1HZH) and $K_V10.1$ (white; PDB5K7L). A single-domain antibody (nanobody, blue; PBD5SV4) is depicted for comparison. The gray band indicates the approximate thickness of the membrane.

because its inhibition underlies a majority of drug-induced long QT events, which can produce malignant arrhythmia and sudden death. The antibody mAb56 was designed and obtained by immunization of mice using a fusion protein that incorporated the E3 region and the tetramerizing coiled-coil domain of K_v10.1, followed by standard hybridoma methodology. mAb56 has a high affinity for K_v10.1, and it is not able to bind to K_v10.2. It inhibits K_v10.1 currents in heterologous and native systems, and it has been demonstrated that mAb56 antibody is capable of inhibiting tumor growth, both in xenograft models of MDA-MB-435S human melanoma cells and in xenografted human pancreatic tumor fragments. Mice treated with a loading dose of 50 mg/kg of the antibody (once measurable tumors had developed), followed by 35 mg/kg weekly,⁹² showed reduced tumor progression in the range of 30–40%. The use of a control anti-K_v10.1 antibody with an intracellular epitope did not modify tumor growth.

This approach has not been successful for other channels. A second look at the structures of both ion channels and antibodies, as sketched in Figure 1, offers an initial explanation for this difficulty. The image depicts K_v10.1, which is actually a relatively large K_v family member, and it is evident that monoclonal antibodies are very large molecules in comparison with ion channels; even moreso if one only takes into account the part of the protein exposed to the extracellular milieu. It is not easy to imagine how such a large molecule as an antibody could get deep enough into the extracellular funnel that leads to the selectivity filter to noticeably interfere with the current.

The initial idea when generating the immunogen in Ref.⁹² was to obtain an antibody that binds close to the channel pore and thereby interferes with ion permeation. Nevertheless, another antibody resulting from the same screening (mAb62), which binds to the same region, 20 residues upstream in the sequence (Fig. 2), did not show any effects on ionic permeation through K_v10.1, which indicates that it is unlikely that the mechanism of inhibition is a direct interference with ion permeation in the channel.

The resolution of the structure of K_v10.1 confirmed the initial suspicion, as the epitope for mAb56 lies far apart from the actual permeation pathway (Fig. 2), and is actually rather close to the epitope for mAb62 (Fig. 2D). The available structure corresponds to a channel in the activated state, but it has the pore gate closed through interaction with calmodulin. It is therefore possible that in the open channel the epitopes face toward the pore. Nevertheless, comparison with the structure of K_v11.1, which is in the open state (Fig. 2E), reveals that the homologous regions still face outward with the gate open. We should point out that this is only an approximation, because parts of the relevant regions are missing in the structure of K_v11.1, and the homology in the area is relatively low; this is actually what makes antibody selectivity possible.

Among the K_v channels under study for therapeutic purposes, K_v1.3 is likely the best studied case because of its relevance for T cell activation and its potential for the management of immune diseases. As a first approach, the blockage of K_v1.3 with antibodies was conducted for

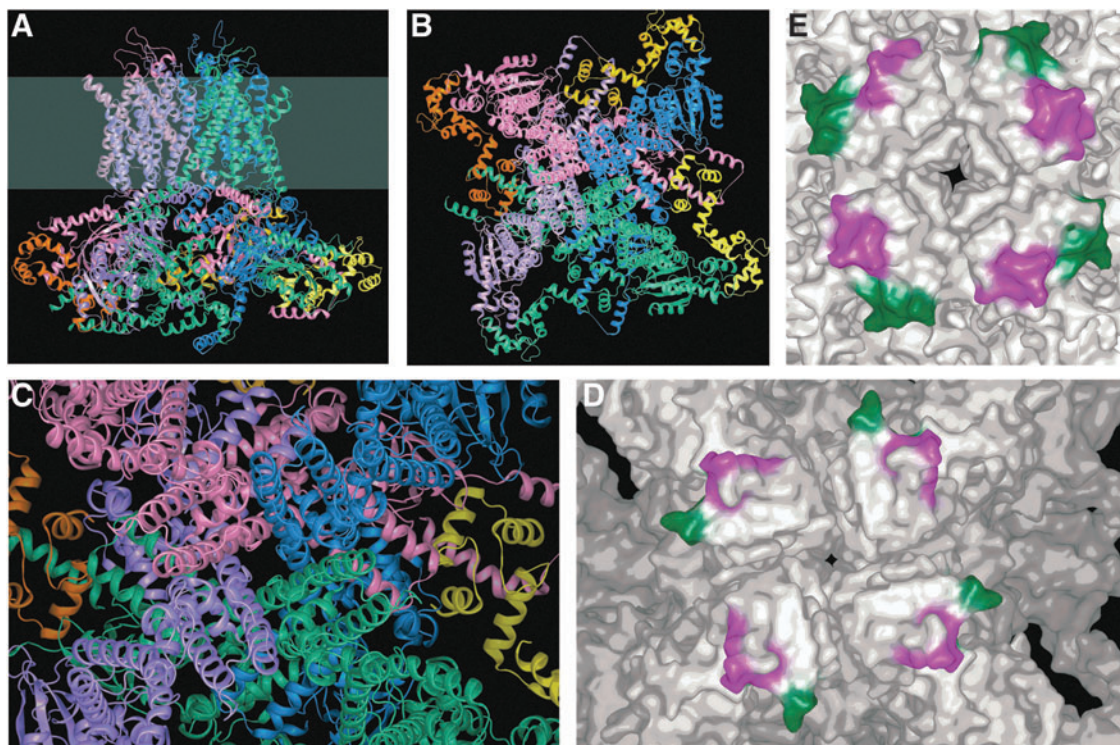


FIG. 2. (A) Cryo-EM structure of K_v10.1 (PDB ID 5k7l) represented as a side view of the tetramer; each monomer is indicated with a different color, and the gray band indicates the plasma membrane (extracellular is represented on the upper part). (B) Depicts the channel seen from the extracellular side at the same scale. (C) and (D) Zoom onto the pore area where mAb56 binds; in the surface representation (D), the epitopes for the blocking antibody (mAb56, magenta) and the non-blocking antibody mAb62 (green) are indicated. Note that both face away from the permeation pathway in the center of the structure. In the structure of the open K_v11.1 (E; PDB ID 5va1), the corresponding regions still face toward the periphery of the channel.

autoimmune and inflammatory diseases such as ulcerative colitis, psoriasis, and type 1 diabetes. A program by Ablynx (a Belgian company now part of Sanofi) used DNA immunization to generate nanobodies, single domain antibodies endogenously found in camelids that are much smaller than regular mAbs. Nanobodies were able to block $K_V1.3$ with low nanomolar affinity and at 10,000-fold higher binding to the closest K_V1 family members, sodium channels, or $K_V11.1$ (HERG). Interestingly, the predominant epitope appears to be the first extracellular loop rather than the pore region.⁹³

A multi-platform approach using purified $K_V1.3$ expressed in *Tetrahymena thermophila* at high levels and reconstituted into liposomes succeeded in generating several functional antibodies, both from chicken (by immunization) and from llama (by a phage display approach), although immunization with whole cells did not produce functional antibodies. The epitopes for the functional antibodies obtained in that study appear to be distributed across all three extracellular loops and are likely nonlinear, further indicating that the mechanism of inhibition exerted by antibodies is more likely to be due to interference with voltage-dependent gating rather than by blocking ionic permeation. The fact that only 1 in 19 nanobodies was functional, compared with 9 of 40 (50 if whole cell immunization is also considered), could also point to this notion, since nanobodies are much smaller but also monovalent and therefore are not able to bind the epitope in 2 of the subunits, thereby interfering with their movements relative to each other.

An alternative approach is the fusion of known active peptides (i.e., toxins) to the complementarity-determining regions of a known antibody specific to a particular channel. This has been carried out successfully in the case of $K_V1.3$,⁹⁴ resulting in a more powerful blockade by the toxin, although it is still unclear if the selectivity achieved comes from the antibody, from the toxin or both.

Despite the difficulty of obtaining functional antibodies, the differential expression of K_V channels in tumor cells offers the possibility of targeting cancer cells based on the surface expression of these channels. Binding or blocking the channels is often not sufficient to eliminate the target cell; therefore, the antibody might better be used as a carrier for an active moiety, such as a cytotoxic drug or a cytokine. We could, for instance, find that a bifunctional antibody consisting of a fusion between an anti- $K_V10.1$ single-chain variable fragment (scFv) antibody and TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) (scFv62-TRAIL; Fig. 3) is able to induce apoptosis not only in tumor cells expressing $K_V10.1$ on their surface but also on the surrounding tumor cells, while leaving nontumor cells unaffected.⁹⁵

The use of scFv62-TRAIL, in combination with the drugs etoposide, roscovitine, and doxorubicin, results in an increase of apoptosis in cancer cells with high endogenous expression of $K_V10.1$,⁷³ providing evidence of the use of antibodies that target potassium channels in cancer as a new and efficient therapy proposal. The effectiveness of combined targeting of a potassium channel and TRAIL can add an additional level of efficacy and specificity to the therapeutic strategy. Although not directly tested for K_V channels, blockade of $K_{Ca}3.1$ by TRAM-34 sensitized melanoma cells to TRAIL treatment.⁹⁶ It is therefore plausible that the fusion of TRAIL to a functional antibody increases the potency of the combined molecule.⁹⁶

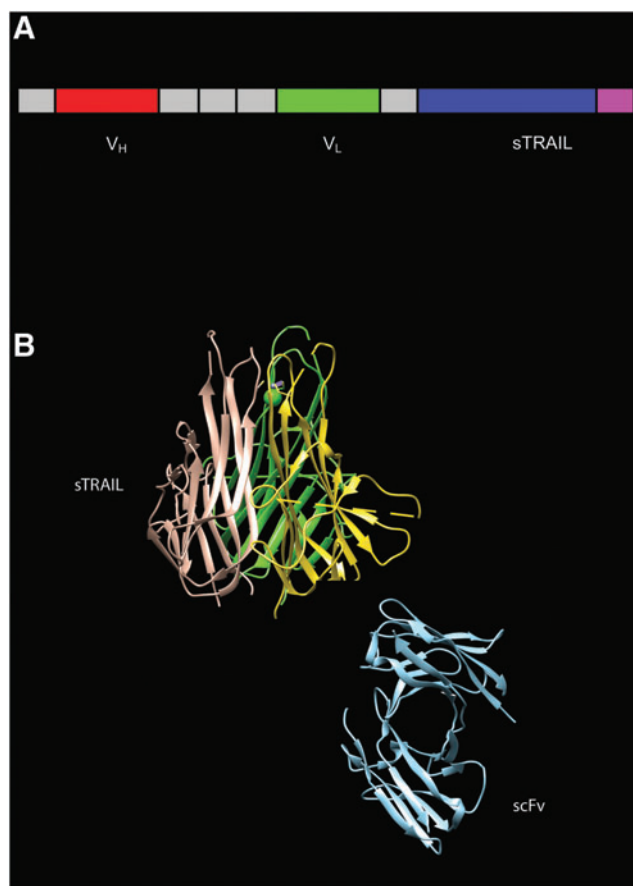


FIG. 3. (A) Structure of the scFv62-TRAIL construct. The fusion consists of an scFv (the variable regions of both heavy [red] and light [green] chains bound by a flexible linker) and the full sequence of soluble TRAIL (purple). The cytokine drives formation of trimers. (B) The structures of a TRAIL trimer (PDB 1DG6) and an scFv monomer (PDB 14PI, tones of blue) are depicted. This is not a structural model of the construct; the molecules have been drawn together only to give an idea of the size of the trimeric construct.

Because $K_V11.1$ (HERG) is an attractive cancer target, its specific blockade in cancer cells would be of interest for therapeutic purposes, but manipulating its activity can result in a high risk of cardiotoxicity. In 2013, the group of Annarosa Arcangeli in Florence synthesized a conjugated of dicarboxylic acid-terminated PEG TiO_2 nanocrystals (PEG- TiO_2 NPs) and anti- $K_V11.1$ to obtain $K_V11.1$ -Mab-PEG- TiO_2 NPs.⁹⁷ This modified antibody recognizes the $K_V11.1$ channel with high specificity in *in vitro* and *in vivo* models of pancreatic adenocarcinoma cancer cells⁸⁹ but does not alter the current nor cause cytotoxicity in cancer cells. Despite its high specificity for $K_V11.1$, this antibody did not cause cytotoxicity in cancer cells; however, it could be used for therapeutic purposes by exploiting the photocatalytic features of TiO_2 with the ensuing ROS production⁹⁸ that would occur upon illumination, or by introducing other new moieties or cytotoxic drugs.

Conclusion

The increasing body of knowledge on both the occurrence and the consequences of the expression of K_V channels in tumors, together with the very detailed information on

structure and function of these molecules and the possibility of detailed molecular studies, makes this family of channels an attractive candidate for the design of personalized therapies for oncological diseases. Although the attempts to generate blocking monoclonal antibodies using conventional approaches have shown a limited success, the insight that structural studies have provided in the last few years makes it possible to design alternative strategies with higher chances of success. This, in combination with the power of protein engineering approaches, allows researchers to combine the advantageous features of antibodies in their many forms with useful features of toxins, cytokines, and other active moieties and will certainly result in improved therapy alternatives in the not too distant future.

Authors' Contribution

All authors contributed to designing the study, collecting and evaluating the information, and writing the article. All co-authors have reviewed and approved the article before submission. The article has been submitted solely to this Journal and is not published, in press, or submitted elsewhere.

Author Disclosure Statement

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