Cancer Biologics Made in Plants

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Abstract

Plants are routinely utilized as efficient production platforms for the development of anti-cancer biologics leading to novel anti-cancer vaccines, immunotherapies, and drug-delivery modalities. Various biosimilar/biobetter antibodies and immunogens based on tumor-associated antigens have been produced and optimized for plant expression. Plant virus nanoparticles, including those derived from cowpea mosaic virus or tobacco mosaic virus in particular have shown promise as immunotherapies stimulating tumor-associated immune cells and as drug carriers delivering conjugated chemotherapeutics effectively to tumors. Advancements have also been made towards the development of lectins that can selectively recognize cancer cells. The ease at which plant systems can be utilized for the production of these products presents an opportunity to further develop novel and exciting anti-cancer biologics.

Graphical abstract

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CONFLICT OF INTERESTS
Nothing declared.

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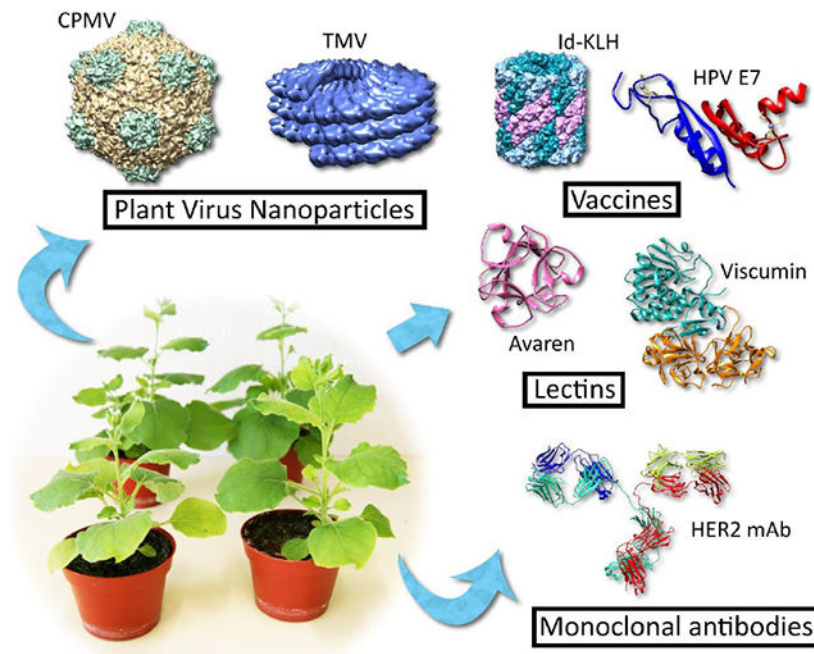
Keywords

Plant-made pharmaceutical; cancer biologic; biopharmaceutical; plant virus; plant virus nanoparticle; therapeutic vaccine; lectin; lectibody; biosimilar; biobetter

INTRODUCTION

The 1997 approval of rituximab, which was the first anti-cancer monoclonal antibody (mAb) approved for use in the U.S., began a biologic explosion that has transformed the landscape of cancer therapy and dramatically altered and improved patient survival and quality of life. According to the U.S. National Cancer Institute (URL: https://www.cancer.gov/about-cancer/treatment/types/immunotherapy/bio-therapies-fact-sheet), this broad category of pharmaceuticals includes immune checkpoint inhibitors, immune cell therapy, therapeutic mAbs and other immune system molecules, therapeutic vaccines and immune system modulators, which now combined make up the majority of total pharmaceutical sales globally (with a market value of approximately 1 trillion dollars in 2016) [1]. Since 1997 hundreds of biologic drugs have been approved or clinically evaluated, and the development of mAbs targeting immune checkpoints like PD-1 and CTLA-4 was even the subject of the 2018 Nobel Prize in Medicine or Physiology, a testament to the impact that biologics have had on medicine. Despite their promise, biologic drugs remain expensive due to manufacturing costs and the lack of significant generic competition from biosimilars (the first was only approved in 2015) [2]. Cell-culture based manufacturing systems are also slow to implement for initial screening and proof-of-concept (POC) studies, prolonging preclinical development of novel drugs, though alternative methods have some important advantages.

Cancer biologic production in plants has a long history, beginning with the early production of mAbs against tumor-associated antigens (TAAs) like CO17-A [3]. In contrast to transgenic plants, the recent advent of transient overexpression vectors allow relatively short time for novel biologic drugs to be produced at scale and tested, making plants an ideal platform for preclinical biologic development [4]. A large number of recent advances in the field have come from the area of plant virus nanoparticles (PVNs), particularly those derived from cowpea mosaic virus (CPMV), tobacco mosaic virus (TMV), and potato virus X (PVX), which have shown efficacy as both immunostimulatory agents/therapeutic vaccines and as drug delivery modalities capable of delivering chemotherapy payloads to tumor sites *in vivo.* While much of the literature is dominated by these advancements, steps have also been made towards the development of recombinant cancer vaccines based on tumor antigens and anti-cancer lectins. This review sets out to catalog recent advancements in plant-made cancer biologics and their future.

CANCER VACCINES AND IMMUNOTHERAPY

The goal of cancer vaccination is to induce tumor-specific immunity and activate immune cells in the tumor microenvironment to elicit anti-cancer activity. Cancer vaccines are immunostimulatory agents that often make use of TAAs, which are antigens capable of distinguishing cancer and non-cancer tissue or antigens that are overexpressed in cancer tissues compared to normal tissue, such as epidermal growth factor receptor (EGFR) and its family in some cancers. One such example is human prostatic acid phosphatase, or PAP, which is a secreted glycoprotein used historically as a marker for prostate cancer. A recombinant PAP fused to granulocyte-macrophage colony-stimulation factor (GM-CSF) is used as a part of spuleucel-T (Provenge®) vaccine, an FDA-approved autologous cellular immunotherapy for prostate cancer [5]. To enhance immunogenicity and expression, Kang et al. has successfully expressed PAP-fused to the Fc region of human IgM in transgenic tobacco *(Nicotiana tabacum)* [6]. Other tumor antigens that have been expressed in plants include the colorectal cancer antigen GA733-2-Fc fusion with an additional KDEL receptor, which was reported to have increased immunotherapeutic effects [7], and idiotypic antibody-keyhole limpet hemocyanin (Id-KLH) conjugate vaccines for Non-Hodgkin's Lymphoma [8–10]. More recently, a phase I safety and immunogenicity trial of Id-KLH conjugate vaccines in 11 patients showed that immunization resulted in a vaccine-induced, idiotype-specific cellular and humoral immune response without any serious adverse events reported [8].

Some chronic infections are known to be risk factors for cancer. A recent report estimated that approximately 15% (2.2 million) of 14 million worldwide new cancer cases in 2012 were attributable to infectious agents, including *Helicobacter pylori,* human papillomavirus (HPV), hepatitis B virus, hepatitis C virus and Epstein-Barr virus [11]. Thus, vaccines against these infections have significant implications for cancer prevention, and a number of efforts have been made for the development of plant-made vaccines against cancer-causing pathogens. However, these vaccines are beyond the scope of this review as they are not strictly categorized as "cancer biologics" with the exception of therapeutic vaccines against HPV E6 and E7 oncoproteins. HPV infection is the cause of approximately 5% of all human cancers, in particular, malignancies of the genitalia (penile, vulval, anal, and cervical

cancers) and oral cavity [11]. The E6 and E7 proteins are ideal targets because they are constitutively expressed in HPV-associated malignant cells and thus may be more effective at generating an immune response to infected cells than L1-based vaccines [12]. One interesting vaccine candidate that has been produced by transient expression in *N. benthamiana* is LALF$_{32-51}$-E7, which is a fusion of the HPV E7 protein to the bacterial cell-penetrating peptide LALF [13]. LALF, or *Limulus polyphemus* anti-lipopolysaccharide factor, can penetrate mammalian cell membranes and has immunomodulatory properties. While plant-made LALF$_{32-51}$-E7 has not been evaluated in animals, it does form the appropriate protein body-like structures in leaf tissue and can be purified to a high degree, and may be a cost-effective therapeutic vaccine candidate [14].

Extensive work in the plant-made pharmaceutical (PMP) research field has been dedicated to the use of PVNs as *in situ* vaccination/immunostimulatory agents with or without the delivery of tumor antigen epitopes, beginning with the first POC study in 2006 with TMV-peptide fusion vaccines [15]. This strategy ultimately aims at the reactivation of tumor-suppressed immune cells in the tumor microenvironment and the induction of systemic anti-cancer immunity. The most well-studied of these PVNs are derived from CPMV, which have demonstrated efficacy in murine 4T1 breast, CT-26, colon, B16F10 melanoma, GL261 glioma, and ID8 ovarian cancer models [16–21]. The icosahedral structure of CPMV appears to be more efficiently taken up by antigen presenting cells (APCs), resulting in higher APC activation and better transport of PVNs to and retention in lymph nodes than high-aspect-ratio viruses like PVX [17,22]. Additionally, as has been recently demonstrated, *in situ* vaccination can result in the conversion of immunosuppressive cells like M2 macrophages and N2 neutrophils to their M1 and N1 counterparts, helping to break tumor immunotolerance [19]. What remains to be seen is the potential efficacy of these particles in humans, as mouse models in these studies used immunodeficient mice for human cancer xenograft, which may have a limited predictive value for immunotherapeutic effects in humans. Nevertheless, the results obtained in recent years hold much promise for their development.

Plants have long been used as production platforms for cancer-targeting immunotherapeutics, including mAbs and cytokines. One such example is the production of CCL21 in tomato, which may potentially be used as an anti-metastatic agent for many cancer types [23]. Recently, several groups have published the production of anti-cancer mAbs including the anti-HER2 mAb trastuzumab [24], the anti-GA733 mAb C017-1A [25], and the anti-CD20 mAbs ofatumumab and rituximab [26,27], as well as an anti-HER2 single chain variable fragment-Fc (scFv-Fc) fusion [28]. Importantly, trastuzumab and rituximab were produced in glycoengineered plants and showed greater anti-cancer activity owing to the lack of core fucose on the single *N*-glycan in the Fc region, which increases the affinity for FcγRIIIa and potently elicits antibody-dependent cell-mediated cytotoxicity [29]. Similar increases in Fc effector functions were also seen for a plant-produced anti-CD20-hIL-2 immunocytokine, made by the fusion of an anti-CD20 mAb and human IL-2 [30]. The resulting immunocytokine was also highly efficient at activating T cells, potentially resulting in greater cytotoxic T cell responses against malignant cells. The relative ease of plant glycoengineering compared to mammalian or insect cell culture systems make them a useful alternative for mAb production [31].

DRUG DELIVERY AND IMAGING

Considerable research has been conducted into novel drug delivery systems, with the goal of improving the pharmacokinetics and pharmacodynamics of small molecule and biologic drugs by affecting their absorption and distribution in the body. PVNs have been particularly attractive owing their ability to deliver larger payloads than antibody-drug conjugates, the relative ease at which they can be decorated with targeting ligands for tissue-specific delivery of drugs, the wide array of possible chemistries, and the ease of manufacturing them *in planta*. So-called high-aspect-ratio viruses, like TMV and PVX, are particularly useful as they are not taken up as easily by phagocytosis and may have a prolonged serum half-life compared to icosahedral viruses. Several recent reports have described PVNs for chemotherapeutic and imaging reagent delivery, including those derived from TMV [32–37], PVX [38–40], red clover necrotic mosaic virus (RCNMV) [41], Pepino mosaic virus (PeMV) [40], and Johnsongrass chlorotic stripe mosaic virus (JgCSMV) [42,43].

Much of the recent work regarding PVNs as drug delivery modalities has focused on the use of high-aspect ratio, or filamentous, viruses. Among these, TMV PVNs have been extensively researched and used in medical imaging and animal models of cancer. Notably, Franke et al. demonstrated that the conjugation of cisplatin to tobacco mosaic virus PVNs restored the efficacy of the chemotherapeutic to OVCAR3 cells, which are typically cisplatin resistant [36]. Because resistance to cisplatin is common among patients with recurrent ovarian cancer [44], the results justify further preclinical validation. Similarly, PVX particles loaded with doxorubicin or presenting tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) have also shown to be efficacious in mice xenografted with the human breast cancer cell line MDA-MB-231 [38,39]. PVX however may have less desirable pharmacokinetic and tumor homing properties compared to another filamentous virus, PeMV [40]. Overall, much work remains to demonstrate how these particles end up at the tumor site and how that can be improved, in addition to further demonstration of efficacy in animal models.

A number of icosahedral viruses have also been used as drug delivery systems, including RCNMV and JgCSMV. While icosahedral virus particles have short serum half-life as efficiently taken up by the immune system, these PVNs appear to have excellent tumor penetration and drug carrying capacity. For example, RCNMV particles loaded with doxorubicin showed more efficacy at a lower dose in an SKOV3 human ovarian cancer xenograft model than the pegylated liposomal form of the drug, indicating a greater degree of tumor targeting by the PVNs [41]. Alemzadeh et al. also recently demonstrated the loading of JgCSMV particles with doxorubicin and their efficacy in the MCF-7 human xenograft breast cancer model in mice [42,43]. Interestingly, encapsulation of drug in JgCSMV particles led to increased uptake of doxorubicin in the breast cancer tissue and actually led to decreased cardiotoxicity [42].

Many recent advances have come in the area of medical imaging. TMV PVNs have, for example, been successfully conjugated to the contrast agent dysprosium and used to image prostate cancer cells in mice using both ultra-high-field magnetic resonance and near-infrared fluorescence imaging [34]. Serum-albumin-coated particles, which may reduce the

potential immune response to the TMV, have also been characterized and used to deliver doxorubicin and the contrast agent gadolinium in mouse models of human breast cancer. Dubbed a "theranostic", this approach successfully combined treatment and MRI imaging, demonstrating the dual capacity of PVNs and their superiority over conventional drug-delivery modalities.

ANTI-CANCER LECTINS

Lectins are a diverse group of carbohydrate-binding proteins that have garnered much interest for their potential immunomodulating and cancer-targeting abilities. In recent years, a great number of fungal and plant lectins with anti-cancer activity have been isolated, characterized, and described in the literature [45,46]. Plant lectins in particular have been historically important as alternative or adjuvant therapies for cancer especially in Europe, where arguably the most well-known is a lectin-containing extract from European mistletoe (*Viscum album*). One of the active ingredients in the extract, viscumin (also called mistletoe lectin or ML), is a holotoxin consisting of a single ribosome-inactivating A chain and a single sialic acid-specific lectin B chain covalently linked with a disulfide bond [47,48]. Though a comprehensive systematic review of the use of mistletoe extract in addition to chemotherapy concluded that it offered no additional benefit in terms of survival or quality of life, isolated viscumin may still have useful anti-cancer activity *in vivo* [49–51]. To this end, Gengenbach et al. recently published the expression and purification of recombinant viscumin in *N. benthamiana*, with a yield of ≈ 7 mg/kg fresh weight. The plant-derived lectin exhibited greater cytotoxicity to THP-1 cells than *E. coli*-made viscumin and was significantly more cost effective to produce [52]. While promising, further *in vitro* and *in vivo* studies are warranted to demonstrate efficacy in multiple models and to demonstrate the selectivity of viscumin for cancer over healthy tissue. Additionally, improvements in yield are necessary to facilitate preclinical development.

Cholera toxin B subunit (CTB) is a non-toxic lectin component of the holotoxin that recognizes the Galβ1–3GalNAc moiety of GM1 ganglioside found on the surface of intestinal epithelial cells (where the toxin normally exerts its activity). We have recently demonstrated that oral administration of a CTB variant (containing a KDEL endoplasmic reticulum retention motif) produced in *N. benthamiana* can facilitate mucosal healing and reduce tumorigenesis in a colitis-associated colorectal cancer mouse model [53]. Epidemiological evidence has pointed to an increase in colorectal cancer incidence in inflammatory bowel disease patients [54,55]. Thus, the plant-made CTB variant as a treatment for chronic intestinal inflammation may also have anti-cancer properties that should be investigated further.

Lastly, aberrant glycosylation has been recognized a hallmark of cancer, and in particular high-mannose glycans have been demonstrated to be over-represented in the glycocalyx of many human cancers [56,57], making them a potentially useful biomarker or druggable target [58]. Our lab has recently developed Avaren-Fc, a plant-produced "lectibody" targeting a cluster of high-mannose glycans that are widely found on the surface of enveloped viruses and malignant cells [59,60]. While originally developed as an anti-HIV agent, we observe that Avaren-Fc also has strong anti-cancer activity *in vitro* and in human

cancer cell xenograft and syngeneic mouse models (Dent and Matoba, unpublished), highlighting the druggability of HIV- and tumor-associated high-mannose glycans.

CONCLUSIONS

Transient expression of proteins in plants is a powerful method for the rapid, robust production of recombinant proteins, which will significantly facilitate the preclinical development of biosimilar, biobetter and innovator anti-cancer proteins as well as vaccines. PVNs show promise as immunostimulatory agents, drug delivery platforms and imaging probes. Since aberrant protein glycosylation is a hallmark of cancer [61,62], plant-derived lectins and their derivatives such as "lectibodies" may have unique potential as cancer biologics.

As regulatory frameworks for plant-based biomanufacturing system are becoming well established [8,63–66], the technology has finally come of age. In addition to transient expression, other technologies based on transgenic plants and plant-cell culture offer some potential advantages that may facilitate the commercialization of plant-made biologics. Transgenic plants, for instance, offer greater scalability and simpler upstream processing, while plant-cell culture is similar to existing platforms that are well-established in the pharmaceutical industry, allowing for the adaptation of conventional chemical engineering and regulatory approaches. Though there are still challenges to be addressed in regard to plant growth conditions, transgene expression regulation, post-translational modifications, and product isolation and recovery, we will soon witness some plant-made cancer biologic products being tested for their clinical efficacy – the most important step that will further cement plants as a viable alternative to other more established production methods.

ACKNOWLEDGEMENTS

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HIGHLIGHTS

- Plant overexpression systems facilitate preclinical biologics development

- Plants have been used to produce many protein vaccines, mAbs and immunotherapeutics

- Plant virus nanoparticles are effective cancer-targeting agents

- Plant-derived lectins and lectibodies may have unique anti-cancer potential

Table 1 –

Recent developments in plant-made cancer biologics. CPMV: cowpea mosaic virus; XF: with plant xylose/fucose deleted glycans; Fc: crystallizable fragment of human antibody; HPV: human papillomavirus; JgCSMV: Johnson grass chlorotic stripe mosaic virus; mAb: monoclonal antibody; PAP: prostatic acid phosphatase; PVX: potato virus X; RCNMV: red clover necrotic mosaic virus; TAA: tumor-associated antigen; TMV: tobacco mosaic virus; TRAIL: tumor necrosis factor-related apoptosis-inducing ligand

Protein	Application	Produced in	Reference
CPMV particles	Immunotherapy, drug delivery	*Vigna unguiculata* (viral propagation) or *N. benthamiana* (transient expression)	[16–21]
TMV particles	Immunotherapy, drug delivery, imaging	*N. benthamiana* (propagation or transient expression)	[16,33–36,40]
PVX particles	Immunotherapy, drug delivery	*N. benthamiana* (viral propagation)	[17,22,38]
PeMV particles	Immunotherapy	*N. benthamiana* (viral propagation)	[40]
TMV-Albumin particles	Immunotherapy, drug delivery	*N. benthamiana* (viral propagation),	[32]
RCNMV particles	Drug delivery	-	[41]
TMV-coated mesoporous silica nanoparticles	Drug delivery	*N. benthamiana* (viral propagation)	[37]
TRAIL–coated PVX	Drug delivery, therapeutic	*N.benthamiana* (viral propagation)	[39]
JgCSMV particles	Drug delivery, therapeutic	*Sorghum halepense* (viral propagation)	[42,43]
PAP-IgM Fc	Immunotherapy	*Nicotiana tabacum* (transgenic, *in vitro* cultured)	[6]
GA733–2-Fc-KDEL	Immunotherapy	*N. benthamiana* (transient expression)	[7]
Oligomannose rituximab	Immunotherapy	*N. benthamiana* (transient expression)	[27]
Ofatumumab	Immunotherapy	*N. benthamiana* (transient expression)	[26]
Trastuzumab	Immunotherapy	*N. benthamiana* (transient expression)	[24]
CO17-1A-KDEL	Immunotherapy	*N. benthamiana* (transient expression)	[25]
Anti-CD20 scFv-Fc-hIL2 immunocytokine (XF)	Therapeutic	*N. benthamiana* (transient expression)	[30]
Anti-HER2 scFv-Fc	Therapeutic	*Arabidopsis thaliana*	[28]
Viscumin (mistletoe lectin)	Therapeutic	*N. benthamiana* (transient expression)	[52]
Avaren-Fc	Therapeutic	*N. benthamiana* (transient expression)	[59]
CTB-KDEL	Immunotherapy	*N. benthamiana* (transient expression)	[53]
LALF$_{32-51}$-E7 HPV vaccine	Therapeutic vaccine	*N. benthamiana* (transient expression)	[13,14]
CCL21	Immunotherapy	*Solanum lycopersicum* (transient expression)	[23]