

# Rapid, high-yield production in plants of individualized idiotype vaccines for non-Hodgkin's lymphoma

M. Bendandi<sup>1</sup>, S. Marillonnet<sup>2</sup>, R. Kandzia<sup>2</sup>, F. Thieme<sup>2</sup>, A. Nickstadt<sup>2</sup>, S. Herz<sup>2</sup>, R. Fröde<sup>2</sup>, S. Inogés<sup>1</sup>, A. López-Díaz de Cerio<sup>1</sup>, E. Soria<sup>1</sup>, H. Villanueva<sup>1</sup>, G. Vancanneyt<sup>3</sup>, A. McCormick<sup>4</sup>, D. Tusé<sup>5</sup>, J. Lenz<sup>6</sup>, J.-E. Butler-Ransohoff<sup>7</sup>, V. Klimyuk<sup>2</sup> & Y. Gleba<sup>2\*</sup>

<sup>1</sup>Laboratory of Immunotherapy, Oncology Division, Center for Applied Medical Research, University of Navarra, Pamplona, Spain; <sup>2</sup>Icon Genetics GmbH, Halle, Germany; <sup>3</sup>Bayer BioScience, Gent, Belgium; <sup>4</sup>Matrix Bioresearch, Vacaville, CA, USA; <sup>5</sup>DT/Consulting Group; <sup>6</sup>Bayer Schering Pharma, Wuppertal, Germany and <sup>7</sup>Bayer Innovation GmbH, Duesseldorf, Germany

Received 17 November 2009; revised 31 March 2010; accepted 2 April 2010

**Background:** Animal and clinical studies with plant-produced single-chain variable fragment lymphoma vaccines have demonstrated specific immunogenicity and safety. However, the expression levels of such fragments were highly variable and required complex engineering of the linkers. Moreover, the downstream processing could not be built around standard methods like protein A affinity capture.

**Design:** We report a novel vaccine manufacturing process, magniflection, devoid of the above-mentioned shortcomings and allowing consistent and efficient expression in plants of whole immunoglobulins (Igs).

**Results:** Full idiotype (Id)-containing IgG molecules of 20 lymphoma patients and 2 mouse lymphoma models were expressed at levels between 0.5 and 4.8 g/kg of leaf biomass. Protein A affinity capture purification yielded antigens of pharmaceutical purity. Several patient Igs produced in plants showed specific cross-reactivity with sera derived from the same patients immunized with hybridoma-produced Id vaccine. Mice vaccinated with plant- or hybridoma-produced Igs showed comparable protection levels in tumor challenge studies.

**Conclusions:** This manufacturing process is reliable and robust, the manufacturing time from biopsy to vaccine is <12 weeks and the expression and purification of antigens require only 2 weeks. The process is also broadly applicable for manufacturing monoclonal antibodies in plants, providing 50- to 1000-fold higher yields than alternative plant expression methods.

**Key words:** idiotype, lymphoma, magniflection, plant-produced, recombinant, vaccine

## introduction

Non-Hodgkin's lymphoma (NHL) is the most common hematologic malignancy in the United States, with an estimated 66 000 new cases this year [1]. Approximately a quarter of these cases are follicular B-cell lymphoma (FL), a disease still considered incurable [2]. Most patients subjected to standard treatments such as chemotherapy, radiation or ablative anti-B-cell antibody therapy still relapse [2]. Although B-cell tumors in general and FL in particular are characterized by extreme variability in treatment and prognosis, they share certain features that make them ideal for the development of patient-specific cancer vaccines. Each clone of malignant B cells expresses a unique cell surface immunoglobulin (Ig), which serves as a tumor-specific marker. Therefore, newer approaches have focused on active immunotherapy through vaccination with the patient's own idiotype (Id) conjugated to a strong immunogen

such as keyhole limpet hemocyanin (KLH) and administered with an adjuvant-like granulocyte-macrophage colony-stimulating factor (GM-CSF). The history of Id vaccination for follicular lymphoma is >20 years old, and numerous studies have demonstrated that upon vaccination during chemotherapy-induced remission, a large percentage of patients develop an antigen-specific immune response [3, 4] and the responders have a superior clinical outcome [5], although these proofs of principle have not yet been confirmed in large studies [6]. In particular, and as predicted [5], all three phase III clinical trials aiming at Id vaccine regulatory approval for follicular lymphoma have failed to achieve their main clinical end points mainly because of serious pitfalls in their study design or in their conduction [6, 7].

Several methods for Ig-based vaccine production have been used, including development of hybridomas that secrete patient's tumor-specific Ig, cloning of Ig variable regions and expression of full Ig molecules in *Escherichia coli*, insect or mammalian cells [8–10] or expression of cloned variable regions as single-chain variable fragments (scFv) in plants using plant viral vectors [11–13].

\*Correspondence to: Dr Y. Gleba, Icon Genetics GmbH, Weinbergweg 22, 06120 Halle, Germany. Tel: +49-345-5559-886; Fax: +49-345-5559-884; E-mail: gleba@icongenetics.de

Rapid manufacturing of Id-specific vaccines in plants using transient expression systems is especially promising. In extensive previous studies conducted by Large Scale Biology Corporation (Vacaville, CA), plant-produced individualized scFv vaccines for the treatment of NHL demonstrated specific immunogenicity and protection in animal challenge studies and safety and immunogenicity in a phase I clinical trial [11–13]. However, the vaccine design based on scFv molecules required complex engineering of the linkers and standard methods such as protein A affinity capture could not be used for purification.

To improve expression levels, and streamline protein purification, we applied magnification, the new transient expression system that allows efficient expression of whole Igs in plants [14–17]. We were able to produce individualized vaccines through a protocol that is robust, rapid and scalable to meet industrial requirements. In our study, each produced vaccine antigen consists of a protein containing the patient's tumor-specific sequence, the Id, intended to induce a specific immunological response leading to complete molecular remission and long-term disease-free survival. To increase its immunogenicity, the antigen is conjugated to subunits of the carrier protein KLH and co-administered with GM-CSF (Leukine®, Genzyme Corporation, Cambridge, MA, USA) as an adjuvant.

## results

### cloning and expression of Igs

Our antigen design comprises an Id-containing IgG obtained by genetically fusing the variable region of the patient's tumor-specific Id with a generic constant domain of a human IgG1, regardless of the original tumor isotype (IgG or IgM). The variable light chain ( $V_L$ ) is fused to a generic kappa or lambda light-chain constant domain, depending on whether the patient's tumor-specific Ig contains a kappa or a lambda light chain.

The patient-obtained biopsies used in this study represented 20 different tumor Igs, 3 of which consisted of an IgG/kappa, 7 IgG/lambda, 7 IgM/kappa and 3 IgM/lambda resulting in 10 analyzed sequences per Ig class. Additionally, two syngeneic mouse lymphoma models, 38C13 (IgM/kappa) and A20 (IgG2a/kappa), were used to compare immunogenicity and protection *in vivo*.

For identification of the patient's tumor-specific Id, an improved molecular cloning procedure was applied in this study, allowing identification of the tumor Id for all (20 of 20) biopsies analyzed. Cloning and identification of each Id are done by amplification of the variable region by anchored polymerase chain reaction (PCR) [18], cloning of the PCR product (which consists of a library of variable regions of Igs expressed in the biopsy sample) and sequencing 96 clones of the library using a vector primer. The PCR using the anchor primer and four different specific primers per Ig class, respectively, were optimized to obtain enough product for cloning after a single amplification. Using this strategy, all sequences could be analyzed without ambiguity. This improved process will be described in detail in a separate publication.

For the magnification process, the variable regions of the Id were then subcloned in magnICON® vectors (Icon Genetics, Halle, Germany) containing a plant signal peptide and a codon-optimized Ig constant region. The variable regions of the heavy and light chains were subcloned in both tobacco mosaic virus (TMV) and potato virus X (PVX) vectors, so that infiltration can be carried out with the heavy chain expressed in TMV and the light chain in PVX and with the opposite orientation. The TMV and PVX expression vectors were transformed into the industrial *Agrobacterium* strain ICF 320, a disarmed, auxotrophic derivative (*DcysK<sub>a</sub>*, *DcysK<sub>b</sub>*, *DthiG*) of *Agrobacterium tumefaciens* strain C58, which was generated for this process. Both construct combinations were then *Agrobacterium*-mediated delivered into *Nicotiana benthamiana* [14, 15] to test for the best possible combination of expression of the Id (data not shown). As the magnification process was repeated several times per each antibody in order to gather enough material for extensive toxicology studies, we were able to systematically confirm the identity of each patient's antibody originated by independent runs of the entire process.

To optimize the level of Ig expression, we also tested the effect of native and heterologous signal peptides derived from secreted proteins of different plant species (e.g. *Nicotiana glauca* calreticulin, barley  $\alpha$ -amylase, rice  $\alpha$ -amylase 3A, bean  $\alpha$ -amylase inhibitor, bean polygalacturonase-inhibitor protein, apple tree pectinase, pea endochitinase A2, *Arabidopsis* auxin-binding protein ABP1) and assessed the effect of these substitutions. The best performance was found with the signal peptides from rice  $\alpha$ -amylase and barley  $\alpha$ -amylase (data not shown). In two cases, significant improvement of expression was achieved by amino acid residue substitution of the first amino acid of the mature protein, proline to serine in the light chain of patient 53 and proline to glutamate in the heavy chain of mouse sample A20. As documented by the matrix-assisted laser desorption/ionization – mass spectrometer tryptic peptide fingerprint, the presence of proline at the N-terminus of the predicted mature Ig chain leads to signal peptide miscleavage at the wrong site and, consequently, to low level of Ig accumulation (data not shown).

The above-mentioned modifications resulted in expression cassettes that provided expression levels for all 22 Igs which were visible on a Coomassie-stained gel run with total soluble protein extracts (Figure 1A). Quantitative enzyme-linked immunosorbent assay (ELISA) analysis gave a range of expression yields between 0.5 and 4.8 g of Ig per kilogram of fresh leaf biomass [leaves constituted (Figure 1B) ~50% of the total aerial biomass of *N. benthamiana* plants grown under our conditions).

Our protocol based on magnification allowed reliable, uniform and high-level expression of 22 (20 human- and 2 mouse-derived) lymphoma-associated Igs processed for vaccine production.

### Ig purification and protein analysis

A highly robust and reliable protocol for Ig purification based on protein A affinity capture has been developed for molecules produced in this study. For Ig purification, 5 kg of leaf biomass was homogenized. Lowering the pH of the plant homogenate

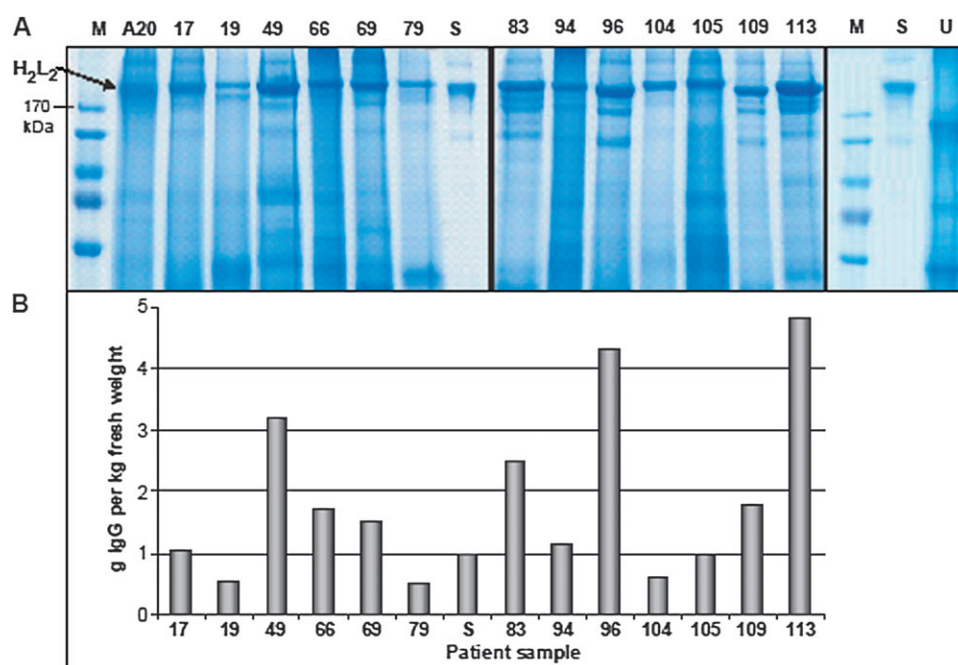


**Figure 1.** Expression levels of various immunoglobulin (Ig) molecules. (A) Coomassie blue-stained 8% polyacrylamide gel separation (nonreducing conditions) of total leaf extracts expressing full IgG molecules cloned from 13 different patients and from A20 lymphoma mouse model. Expression levels of the remaining eight samples not shown were comparable. The lane numbers correspond to the patient codes. Lane A20, mouse lymphoma Ig. Lane S, human IgG1/kappa standard. Lane M, 40–170 kDa marker proteins, the 170 kDa protein indicated, lane U protein extract from a non-infiltrated *N. benthamiana* plant. (B) Results of enzyme-linked immunosorbent assay measurements of the expression levels of IgG molecules for the same patients. The yield was calculated in grams of IgG protein per kilogram of fresh leaf biomass.

to <5.1 resulted in removal of many highly abundant host cell proteins, e.g. Rubisco, and larger debris were filtered out to obtain a crude extract suitable for subsequent chromatography. After protein A affinity chromatography, the Ig-containing eluate was further purified by membrane adsorption chromatography (Figure 2).

Using this protocol, we were able to reliably purify Igs from all patients (including the six for whom Id production from hybridoma had repeatedly failed) and animal sources, with purity levels exceeding 90% as analyzed by capillary gel electrophoresis (Figure 3A) in reduced mode and overall recovery yields >50% (Figures 2 and 3). For calculation of the Ig recovery, the concentrations of Ig in crude leaf extract and at different stages of purification (Ig intermediates) were measured by ELISA (see Materials and Methods section, available at *Annals of Oncology* online). These results reflect the typical robustness of protein A-based affinity capture and the generally high accumulation of Igs in the source plant tissue.

Analysis of the reduced Igs by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and capillary gel electrophoresis revealed for most Ids a pattern for heavy and light chain consisting of several molecules differing in molecular mass. Especially, light chain molecules formed species with different migration behavior (Figure 3A and B). Such patterns are in most cases the result of post-translational modification of the protein, e.g. glycosylation. Therefore, glycosylation was analyzed by immunoblotting using a mixture of anti-fucose and anti-xylose antibodies for detection. As expected, all Ig molecules analyzed so far were glycosylated

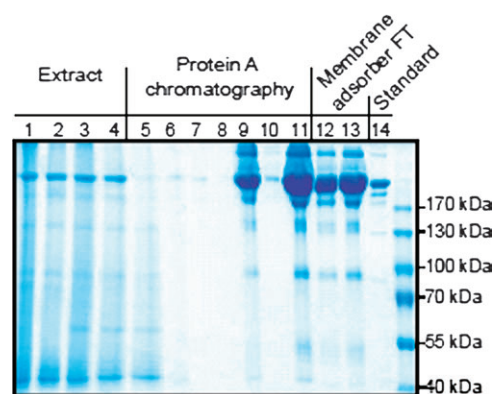


**Figure 2.** Extraction and purification of immunoglobulin G (IgG) from patient 69 under good manufacturing practice-like conditions. Coomassie blue-stained 8% polyacrylamide gel separation (nonreducing conditions): lanes 1–3, crude extracts at different stages of processing; lane 4, filtrated extract; lane 5, flow through from protein A column; lines 6–8, wash fractions; lanes 9 and 11, eluate (peak fractions); lane 10, eluate, side fractions; lane 12, 5  $\mu$ l anion exchange membrane adsorber eluate flow through; lane 13, 11  $\mu$ l anion exchange membrane adsorber flow through and lane 14, 1  $\mu$ g human IgG1/kappa standard. The right lane contains standard molecular markers 40, 55, 70, 100, 130 and 170 kDa.

(Figure 3C), said glycosylation causing most if not all of the heterogeneity of the Igs studied. In contrast to light chains, heavy chains show more consistent glycosylation due to location of the respective sites within the constant region of IgG1 which was used for all constructs.

N-terminal sequencing of the light and heavy chain revealed correct cleavage of analyzed mature Igs. However, sequencing could not be carried out for N-termini of ~40% of the Igs because of predicted inaccessibility due to pyroglutamate formation. In addition, MALDI-MS peptide fingerprinting and comparison with theoretically expected peptides for 12 Igs confirmed protein identity and proper signal peptide cleavage in optimized versions (data not shown), also indicating that heterogeneity of the Igs is based on post-translational modifications rather than on miscleavage.

### cross-reactivity studies

The aim of this analysis was to assess cross-reactivity of patient-specific hybridoma- and plant-produced Igs with the sera of vaccinated patients. Patient sera were obtained after vaccination with tumor-derived Ids, which were rescued using a hybridoma-based strategy [19, 20]. The cross-reactivity of hybridoma- and magnICON®-produced Igs with the sera of FL patients was assessed by ELISA [3, 4, 21].

A couple of representative results of such cross-reactivity are provided in Figure 4. Most promisingly, despite the differences (e.g. different glycosylation patterns, different heavy-chain

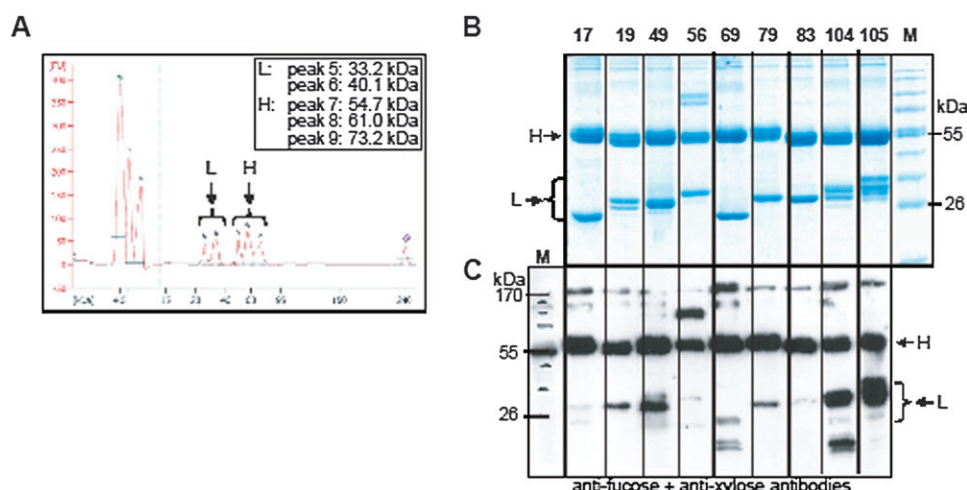


**Figure 3.** Glycan detection on heavy and light chains of selected patient immunoglobulin G (IgG) molecules. (A) Capillary gel electrophoretic separation (reducing conditions) of IgG molecules cloned from patient 19. The differences between theoretical and determined molecular weight is caused by migration alteration due to glycosylation. (B) Coomassie blue-stained 12% polyacrylamide gel separation (reducing conditions) of purified IgG molecules cloned from nine different patients. The lane numbers correspond to the patient codes. The right lane is standard molecular 26–170 kDa markers and 26 and 55 kDa marker proteins are indicated. (C) Immunoblot analysis of the same immunoglobulins as in (B) using a mixture of anti-fucose and anti-xylose antibodies. Heavy and light chains are indicated as H and L, respectively. The left lane is standard molecular markers.

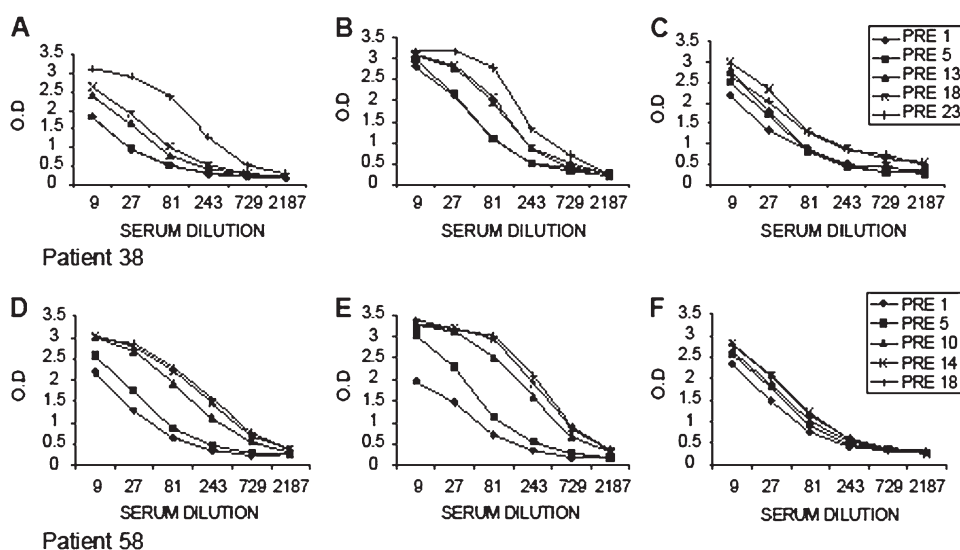


**Figure 4.** Cross-reactivity between hybridoma- and magnICON®-derived idiotypes (Ids). The cross-reactivity is exemplified by two cases (patient 38: A through C; patient 58: D through F). (A, D) Post-vaccine sera-specific recognition of the hybridoma-derived Ids actually used to vaccinate these two patients. (B, E) Post-vaccine sera-specific recognition of the corresponding magnICON®-derived Ids never used to vaccinate these two patients. (C, F) Lack of substantial recognition of irrelevant, isotype-matched Id controls by the post-vaccine sera. O.D., optical density; Pre-1, titer of pre-immune serum; Pre-5: titers using serum before fifth vaccination; subsequent numbers indicate the number of vaccinations before serum retrieval.

constant region in patients with tumors expressing an Id-containing IgM) between the hybridoma-made Id used for vaccination and the plant-made Id which had never been used to immunize those patients, in four cases of nine tested post-vaccine sera from patients recognized both Id. In particular, we observed cross-reactivity in four of five cases featuring an Id-containing tumor IgG and in zero of four cases featuring an Id-containing tumor IgM (data not shown).

### tumor challenge studies *in vivo* with murine lymphoma models

Both the 38C13 and the A20 models were used. The goal of the study with the former model was to determine the immunogenicity of a plant-produced 38C13 IgM-KLH vaccine (50 and 10 µg dose per immunization) administered with the adjuvant GM-CSF compared with a negative control consisting of co-administered KLH + GM-CSF in phosphate-buffered saline (PBS) and a positive control consisting of standard hybridoma-produced IgM-KLH vaccine.

Mice were vaccinated with Id-KLH conjugates by subcutaneous delivery in combination with GM-CSF. Anti-Id antibody responses were measured in serum in all vaccine groups after two immunizations. Results of mouse tumor challenge studies conducted with the 38C13 syngeneic murine lymphoma model are shown in Figure 5. Relative levels of anti-38C13 IgG (1 U is ~1 µg/ml of anti-Id titer) are shown for all vaccine groups, including PBS procedural/vehicle control, KLH carrier protein control, plant-produced IgG at 50 and 10 µg dose per immunization and standard-hybridoma-produced IgM vaccine administered at 50 µg dose (Figure 5A). No detectable anti-38C13 was present in the control PBS or KLH

vaccine groups. Relative levels of IgG1 and IgG2 were also determined for all 38C13 vaccine groups (data not shown).

There was no statistical segregation between the 50-µg-dose groups, of plant- and hybridoma-produced vaccines, showing that the protection afforded by the plant-made vaccine matched that of the standard-produced IgM vaccine (Figure 5B). All vaccine groups statistically differed from the PBS and KLH controls ( $P < 0.0001$ ). The 10-µg group was not significantly different from the 50-µg IgM-KLH vaccine group (data not plotted). In this study, plant-produced, magnICON<sup>®</sup>-derived Id vaccines induced tumor-specific protection in animal challenge studies in a dose-dependent manner.

The goal of the study with the latter model was to determine the protective ability of a plant-produced A20 IgG1-KLH vaccine (50 µg dose per immunization) administered with the adjuvant GM-CSF compared with a negative control consisting of co-administered KLH + GM-CSF in PBS and a positive control consisting of animal cell culture-produced IgG2a-KLH vaccine.

Mice were vaccinated with Id-KLH conjugates by subcutaneous delivery in combination with GM-CSF. Results of mouse tumor challenge studies conducted with the A20 syngeneic murine lymphoma model are shown in Figure 6. Both vaccine groups statistically differed from the PBS and KLH controls ( $P < 0.0001$ ).

### discussion

The approach described in this study represents a dramatic improvement in the efficiency of producing Id-based vaccines in plants, due to the level and speed of antigen expression in the

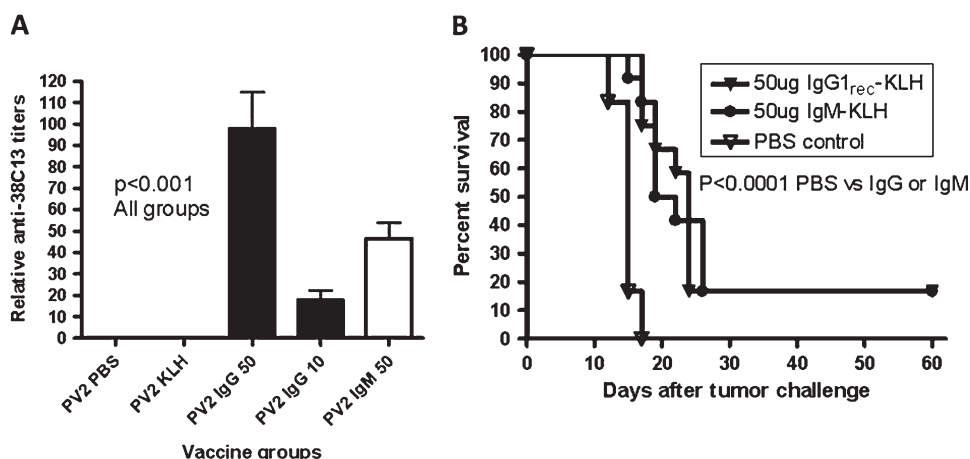


**Figure 5.** Plant-produced 38C13 immunoglobulin (Ig) immunogenicity in animal challenge model. (A) Anti-38C13 titers in vaccinated animals. Ten days after the second vaccination, serum from each group of 12 C3H vaccinated mice was collected and analyzed by 38C13 scFv sandwich enzyme-linked immunosorbent assay. Relative levels of IgG are shown for all vaccine groups (1 U is ~1 µg/ml). No detectable anti-38C13 was present in the phosphate-buffered saline (PBS; buffer/vehicle control) or keyhole limpet hemocyanin (KLH; carrier protein control) groups. (B) Mice immunized with the magnICON<sup>®</sup> plant-produced 38C13 IgG1-KLH protein conjugate are protected from tumor challenge. Mice were immunized two times with 10 (data not shown) or 50 µg Ig-KLH plant-made antigens or 50 µg IgM-KLH-positive control antigen ('gold standard'). Twelve days after the last immunization, mice were challenged by subcutaneous injection of  $1.5 \times 10^3$  38C13 tumor cells. When tumor size reached 2.0 cm (length  $\times$  width), mice were killed and day of killing was recorded. Protection from tumor progression was estimated from the relative rate of growth of the tumor from tumor implantation (day 0) to the date of killing, in each animal by dose group. Data were analyzed by Kaplan–Meier analysis and plotted as percent survival. The results shown are representative of three experiments. All groups received the same amount of granulocyte–macrophage colony-stimulating factor.

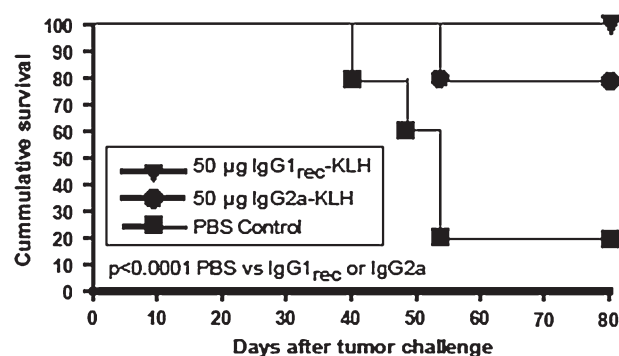


**Figure 6.** Plant-produced A20 immunoglobulin (Ig) protection in animal challenge model. Mice immunized with the magnICON<sup>®</sup> plant-produced A20 IgG1-keyhole limpet hemocyanin (KLH) protein conjugate are protected from tumor challenge. Mice were immunized two times with 50 µg idiotype-KLH plant-made antigens or 50 µg IgG2a-KLH-positive control antigen. Twelve days after the last immunization, mice were challenged by subcutaneous injection of  $5 \times 10^5$  A20 tumor cells. When tumor size reached 2.0 cm (length  $\times$  width), mice were killed and day of killing recorded. Protection from tumor progression was estimated from the relative rate of growth of the tumor from tumor implantation (day 0) to the date of killing, in each animal by dose group. Data were analyzed by Kaplan–Meier analysis and plotted as percent survival. The results shown are representative of three experiments. All groups received the same amount of granulocyte–macrophage colony-stimulating factor.

magnICON<sup>®</sup> system and the ease of isolation of whole Igs using affinity capture.

The magnification-based transient system described yielded reproducibly high and commercially viable levels of 0.5–4.8 g antigen per kilogram of leaf biomass. Those levels have been achieved for all 20 human Igs attempted after a single round of expression optimization in which a number of plant signal peptides were screened in two different combinations of expression vectors (light chain expressed from TMV vector and heavy chain expressed from PVX vector, and *vice versa*). These initial studies allowed us to rapidly narrow down the list of candidate plant signal peptides appropriate for expressing chains of Igs from 15 to just 2 (barley amylase and rice amylase). Together with eight other Igs previously expressed in plants using the magnification method [15], the present study provides clear evidence that plants are capable of efficiently expressing and assembling functional Igs of human or murine origin. The yields of scFv molecules reported in a previous study [11], on the contrary, were lower and highly variable. Of the 44 scFv molecules expressed in plants using viral vectors, 6 failed to express a Coomassie stainable band from crude extracts, and the expression variability among those clones with detectable expression was 1000-fold (between 0–4 and 100–800 mg/l of interstitial fluid, equivalent to between 0–2 and 50–400 mg/kg of plant biomass).

At least several of the patient-specific Igs in the current study were expressed at levels of up to 4.8 g/kg of leaf biomass or >70% of total soluble protein; such expression levels are close to theoretical limits and have earlier been achieved only with simpler proteins [14, 17, 22], whereas yields of full Igs produced in plants using alternative technologies are 50–1000 times lower (see, for example [23, 24]).

Theoretically, taking into account Id vaccine doses used so far in clinical trials [7], an NHL patient might need <25 mg of Ig for a lifetime of therapy, which amount, together with ~200 mg needed for in-process control, release and stability testing plus required retain samples, could be easily produced from <1 kg of plant biomass. In our prototype industrial process, we transfected ~5 kg plant biomass per vaccine candidate (corresponding to ~2.5 kg leaf biomass) because the process is intended for high-throughput manufacturing of vaccines and is not necessarily optimized for the highest expression levels of each antigen. Thus, 5 kg of starting biomass ensures that all vaccine candidates can be produced in clinically relevant amounts.

The use of magnification as an expression system enables a significant reduction in antigen production time. Using transient but non-magnification-based plant viral vectors, McCormick et al. [12, 13] reported elapsed times of ~4 weeks from molecular cloning to identifying the antigen by ELISA and western analyses. By comparison, our transient expression process requires only 2 weeks.

The use of a standardized purification process relying on protein A affinity capture [25] allowed in all cases a reliable Ig purification, and the molecular analysis of the purified proteins demonstrated proper cleavage of plant signal peptide in the majority of cases. Replacing unusual amino acids and those likely to impair cleavage efficiency of the variable region (such as proline) by the amino acid closest to the consensus for that class of variable region resulted in precise cleavage in all cases. Tryptic digest analyses carried out on 12 Igs demonstrated identity of the proteins expressed from viral vectors.

As expected, all Igs tested to date have shown extensive glycosylation, and most molecules had sugars attached to both the constant as well as the variable regions. Yet, the different patterns of glycosylation existing between human FL cells [26, 27], mouse/human heterohybridomas and tobacco plants [28, 29] do not seem to negatively affect overall Id immunogenicity or the cross-reactivity with post-vaccine sera from patients who had developed an Id-specific humoral response as a result of vaccination with their customized, hybridoma-derived and FL-specific Id. In any case, only through clinical trials physically using plant-derived Id vaccines it will be possible to ascertain whether different glycosylation patterns or heavy chains in the vaccine formulation may result detrimental, enhancing or irrelevant. The first of such trials is set to start patient enrollment in the United States within the first trimester of 2010.

As evidenced by the antibody responses in vaccinated C3H/HeN mice as well as from survival data (from both 38C13 and A20 models) in a lethal tumor challenge, the conformation of the plant-made protein is relevant to the tumor Id. The protection afforded by ICON's plant-made vaccines matched that of the corresponding standard Ids, i.e. the vaccine purified from hybridoma cells or animal cell cultures. It is worth mentioning that unlike the 38C13 scFv material used in challenge studies by McCormick et al. [12], the full Igs used in our mouse experiments were glycosylated, thus providing evidence for the first time that glycosylation in plants does not necessarily lower immunization-induced protection against tumor in vaccinated mice.

In conclusion, magniflection is a very rapid and reproducible method for producing clinically relevant amounts of B-cell tumor-specific, Id-containing Igs. In our hands, full IgG molecules representing the Ids of 20 NHL patients [21, 30] have all been efficiently expressed at levels between 0.5 and 4.8 g/kg of leaf biomass [31]. Purification based on industry standard affinity capture yielded materials of acceptable pharmaceutical-grade purity [32, 33]. Although extensively glycosylated, the plant-made Igs of some FL patients showed substantial cross-reactivity with specific sera derived from the same patients solely immunized with the hybridoma-derived vaccine [34]. Moreover, mice vaccinated with either plant- or hybridoma-derived Igs showed, despite their different glycosylation pattern, the same level of protection in tumor challenge studies. The described manufacturing process is reliable and robust; the total manufacturing time starting from biopsy to a conjugated vaccine is <12 weeks and the expression and purification of antigen require only 2 weeks. The methodology described lends itself to the rapid production of individualized proteins, such as Id NHL vaccine antigens, as well as to the prototyping and production of other antigens whose seasonal or mutational variability favor a rapid and flexible manufacturing platform. Together with our earlier findings [15], this process also represents a broadly applicable, robust, scalable and cost-effective platform for manufacturing monoclonal antibodies in plants.

## funding

Icon Genetics GmbH, a wholly owned subsidiary of Bayer AG. Unión Temporal de Empresas project Fundación para la Investigación Médica Aplicada to the work at the Center for Applied Medical Research.

## acknowledgements

We wish to thank Duc Bui Minh, Anatoli Giritch, Franziska Jarczowski, Anne-Katrin Paschke, Yuri Symonenko, and Stefan Werner for their expert help. Icon Genetics GmbH played an active role in study design, data collection and analysis, decision to publish and preparation of the manuscript. The funders have sponsored this research as a general feasibility study. No external grants were used to support this research.

## disclosure

The authors declare no conflict of interest.

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