

InvivoGen Insight

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REVIEW

Improving the efficacy of clinical antibodies

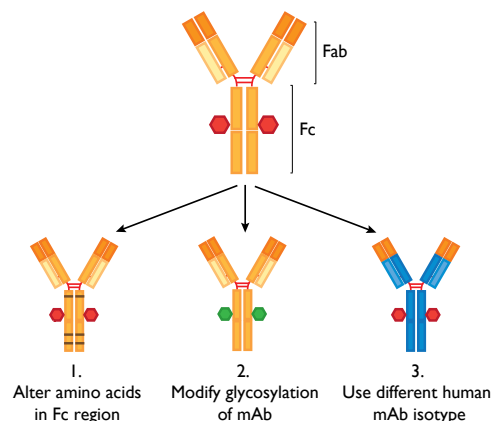
Monoclonal antibodies (mAbs) have shown considerable success as cancer therapeutics and the number of anticancer mAbs in clinical use or under investigation has dramatically increased in recent years. However, there remains much room for improvement. Therapeutic antibodies act mainly by directly targeting tumor cells or by targeting immune cells in the tumor microenvironment. Their ability to induce tumor regression depends on their bifunctional nature: the fragment of antigen binding (Fab) confers antigen specificity, whereas the constant fragment (Fc) triggers antibody-dependent effector functions by engaging a variety of Fc receptors (FcRs). These effector functions include antibody-dependent cellular cytotoxicity and phagocytosis (ADCC and ADCP), and complement-dependent cytotoxicity (CDC), which can combine to facilitate antibody-mediated killing of tumor cells.

Clinical antibodies are of the IgG class, which is divided into four isotypes: IgG1, IgG2, IgG3 and IgG4. The Fc region of these four human IgG isotypes bind to FcRs, with varying affinity and specificity. FcRs are located on the surface of immune effector cells (natural killer cells, neutrophils, macrophages/monocytes) and are either activating (FcγR1, FcγRIIA, FcγRIII) or inhibitory (FcγRIIB). IgG2 and IgG4 interact poorly with FcRs and exhibit weak effector functions, whereas IgG1 and IgG3 interact more strongly with FcRs and thus, mediate potent effector functions. Notably, IgG1 is the most frequently used isotype for anticancer mAbs, as it is the most effective IgG isotype at mediating ADCC. The IgG Fc-FcR binding affinities are determined by the amino acid sequences of the different IgG Fc isotypes and the N-linked glycan patterns of the IgG Fc domains. Thus, the antibody-mediated effector functions of a given antibody can be modulated by engineering its IgG Fc domain to modify the engagement of selected FcRs.

Therapeutic mAbs that target antigens on the surface of malignant cells, such as rituximab (anti-CD20), trastuzumab (anti-HER2) and cetuximab (anti-EGFR), function largely through immune-mediated mechanisms, including ADCC and ADCP¹. Thus, a strategy to improve the efficacy of anticancer mAbs is to augment these effector functions by engineering the IgG1 Fc domain. This can be achieved by changing the amino acid sequence to increase the interaction of the Fc domain with FcγRIII (CD16), as in the case of ocaratuzumab, an anti-CD20 mAb². Further, modification of specific carbohydrates, such as decreased fucosylation of the Fc glycan of IgG1 antibodies, leads to increased affinity for CD16 and consequently, to greater

induction of ADCC/ADCP. Examples of such afucosylated antibodies include obinutuzumab (GA101), an anti-CD20 antibody currently in clinical trials³, and imgatuzumab (GA201), an anti-EGFR antibody under investigation⁴. In addition to IgG1 antibodies, other isotypes induce ADCC/ADCP, such as IgA antibodies, which contribute significantly to the humoral part of the mucosal immune system. Studies exploring their potential have been reported revealing interesting properties of IgA antibodies^{5,6}.

Strategies to improve mAb efficacy



Over the past decade, mAbs that interfere with T cell regulatory pathways have been developed and are generating considerable excitement. These mAbs, called checkpoint blockade antibodies, target regulatory molecules, such as CTLA4 (e.g. ipilimumab) and PD1 (e.g. nivolumab) or its ligand PD-L1 (e.g. atezolizumab), all of which are expressed by immune cells. Nivolumab, originally an IgG1 antibody, is an engineered therapeutic mAb with an IgG4 Fc domain to reduce immune effector functions⁷. Atezolizumab is also an engineered mAb by introduction of a mutation in the IgG1 Fc domain in order to abolish its effector functions⁸.

It is now clear that engineering Fc domains to optimize or minimize their interactions with FcRs will lead to therapeutic antibodies with enhanced antitumor activities.

1. DiLillo DJ, & Ravetch J. 2015. Fc-Receptor Interactions Regulate Both Cytotoxic and Immunomodulatory Therapeutic Antibody Effector Functions. *Cancer Immunol Res.* 3(7):704-13. 2. Cheney CM, et al., 2014. Ocaratuzumab, an Fc-engineered antibody demonstrates enhanced antibody-dependent cell-mediated cytotoxicity in chronic lymphocytic leukemia. *MAbs.* 6(3):749-55. 3. Golay J, et al., 2013. Glycoengineered CD20 antibody obinutuzumab activates neutrophils and mediates phagocytosis through CD16b more efficiently than rituximab. *Blood.* 122(20):3482-91. 4. Gonzalez-Nicolini V, et al., 2015. Premedication and chemotherapy agents do not impair imgatuzumab- (GA201) mediated antibody-dependent cellular cytotoxicity and combination therapies enhance efficacy. *Clin Cancer Res. (Ahead of print).* 5. Lohse S, et al., 2011. Recombinant dimeric IgA antibodies against the epidermal growth factor receptor mediate effective tumor cell killing. *J Immunol.* 186(6):3770-8. 6. Pascal V, et al., 2012. Anti-CD20 IgA can protect mice against lymphoma development: evaluation of the direct impact of IgA and cytotoxic effector recruitment on CD20 target cells. *Haematologica.* 97(11):1686-94. 7. Wang C, et al., 2014. In vitro characterization of the anti-PD-1 antibody nivolumab, BMS-936558, and in vivo toxicology in non-human primates. *Cancer Immunol Res.* 2(9):846-56. 8. Powles T, et al., 2014. MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. *Nature.* 515(7528):558-62.

Antibody Isotype Families

An expanding collection of clinically relevant antibodies

Monoclonal antibodies (mAbs) have become a major tool in cancer therapy. They function through various mechanisms with the ultimate effect of priming either the innate or adaptive arm of the immune system to target tumor cells for destruction. The efficacy of antibodies is governed by their bifunctional nature: the variable region confers antigen specificity and the constant region triggers antibody-mediated effector functions by engaging a variety of Fc receptors. These effector functions include complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP). One approach employed to enhance the efficacy of therapeutic antibodies is to modify the immunoglobulin (Ig) constant region. InvivoGen provides a series of clinically relevant antibodies available in their original format or with a different Ig isotype. The Ig isotypes chosen are human IgG1, IgG4 (S228P) and IgA. These isotypes exhibit distinct properties and therefore, differ in their suitability for a given application.

- Human IgG1: the preferred isotype for antitumor antibodies

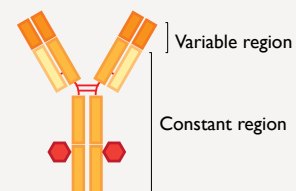
IgG1 is the most abundant Ig isotype in human serum. Human IgG1 Abs lead to high levels of ADCC, ADCP and CDC inducing the death of target cells.

- Human IgG4 (S228P): the preferred isotype for immunomodulatory antibodies

Human IgG4 Abs display reduced ADCC, ADCP and no CDC. These molecules undergo a process known as Fab arm exchange that potentially reduces their therapeutic efficacy. IgG4 (S228P) contains an engineered hinge region mutation (S228P) designed to prevent exchange of IgG4 molecules.

- Human IgA2: an alternative isotype for antibody therapy

IgA is the second most abundant Ig isotype in human circulation and the most prevalent in mucosal secretions. IgA Abs bind to the Fc α RI receptor (CD89), which triggers neutrophil-mediated ADCC and macrophage-mediated ADCP.



	IgG1	IgG4 (S228P)	IgA2
ADCC	++	+/-	+
ADCP	+++	+	+
CDC	+	-	-

InvivoGen's clinically relevant antibodies are generated by recombinant DNA technology, produced in CHO cells and purified by affinity chromatography. They are validated by flow cytometry, are sterile and contain no sodium azide. They are provided in 100- μ g units; however, larger quantities are available upon request.

Anti-hCD20 isotype collection - Rituximab-derived mAbs

The Anti-hCD20 isotype collection features the variable region of rituximab. Rituximab is a chimeric mouse/human IgG1 mAb that targets the CD20 antigen found on the surface of malignant and normal B lymphocytes. Binding of rituximab to CD20 results in cell destruction by inducing apoptosis, CDC and ADCC. The Anti-hCD20 isotype collection comprises 11 additional rituximab-derived mAbs (for a full list, consult the InvivoGen website).

Anti-hEGFR isotype family - Cetuximab-derived mAbs

The Anti-hEGFR isotype family features the variable region of cetuximab. Cetuximab is a chimeric mouse/human IgG1 mAb that targets EGFR, a cell surface receptor overexpressed in many types of cancer. Binding of cetuximab to EGFR blocks ligand/receptor binding and induces receptor internalization and subsequent degradation.

Anti-HER2 isotype family - Trastuzumab-derived mAbs

The Anti-HER2 isotype family features the variable region of trastuzumab. Trastuzumab is a humanized IgG1 mAb that targets the HER2 receptor (HER2/neu, ERBB2), which is over-expressed in certain types of cancers, particularly in breast and ovarian cancers. Binding of trastuzumab to HER2 results in cell death through different mechanisms including ADCC and ADCP.

Anti-hPDI isotype family - Nivolumab-derived mAbs

The Anti-hPDI isotype family features the variable region of nivolumab. Nivolumab is a fully human IgG4 (S228P) mAb that targets the programmed cell death 1 (PD-1) receptor found on activated T cells, B cells and myeloid cells. Nivolumab binds to, and blocks the activation of the PD-1 receptor, which results in activation of T cells and cell-mediated immune responses.

ALSO AVAILABLE: Anti-hCTLA4 (ipilimumab-derived mAbs), and anti-hVEGF (bevacizumab-derived mAbs) isotype families, and anti-hPDL1-mIgG1 InvivoFit™ (atezolizumab-derived mAb). For more information, visit the InvivoGen website.

PRODUCT	ISOTYPE	CAT. CODE
Anti-hCD20 isotype collection (100 μg)		
Anti-hCD20-hIgG1	Human IgG1	hcd20-mab1
Anti-hCD20-hIgG4 (S228P)	Human IgG4 (S228P)	hcd20-mab14
Anti-hCD20-hIgA2	Human IgA2	hcd20-mab7
Anti-hEGFR isotype family (100 μg) NEW		
Anti-hEGFR-hIgG1	Human IgG1	hegfr-mab1
Anti-hEGFR-hIgG4 (S228P)	Human IgG4 (S228P)	hegfr-mab14
Anti-hEGFR-hIgA2	Human IgA2	hegfr-mab7
Anti-HER2 isotype family (100 μg) NEW		
Anti-HER2-Tra-hIgG1	Human IgG1	her2tra-mab1
Anti-HER2-Tra-hIgG4 (S228P)	Human IgG4 (S228P)	her2tra-mab14
Anti-HER2-Tra-hIgA2	Human IgA2	her2tra-mab7
Anti-hPDI isotype family (100 μg) NEW		
Anti-hPDI-Ni-hIgG1	Human IgG1	hpd1ni-mab1
Anti-hPDI-Ni-hIgG4 (S228P)	Human IgG4 (S228P)	hpd1ni-mab14
Anti-hPDI-Ni-hIgA2	Human IgA2	hpd1ni-mab7

Anti-hPDL1-mIgG1 InvivoFit™ is an atezolizumab-derived mAb designed for mouse studies. It features the variable region of atezolizumab, a therapeutic mAb that targets human programmed cell death ligand 1 (PD-L1), blocking the interaction with its receptor PD-1. In contrast to nivolumab, which only targets human PD-1, atezolizumab also targets mouse PD-L1. To enable studies in mice, the human effectorless Fc domain of atezolizumab has been replaced by the Fc domain of mouse IgG1, an isotype exhibiting low CDC and no ADCC. In addition, anti-hPDL1-mIgG1 InvivoFit™ is guaranteed sterile and endotoxin-free (<1 EU/mg) and is provided in 5-mg units.

Knockout Reporter Cell Lines

A collection of knockout cells to study interferon responses to nucleic acids

InvivoGen provides a series of knockout (KO) cell lines generated by gene editing from two popular cellular lines: **RAW 264.7** mouse macrophage and **THP-1** human monocyte. The genes targeted for KO are involved in the **interferon (IFN) response to nucleic acids**, such as double-stranded DNA or RNA, and cyclic dinucleotides (CDNs). These genes include those that encode the cytosolic DNA sensor cGAS, the cytosolic RNA sensor RIG-I and the cytosolic CDN sensor STING. Coupled with their parental cell lines, InvivoGen's KO cell lines are invaluable tools for studying the function of these genes or screening for molecules that activate these pathways. To facilitate their study, these cell lines express an IFN-inducible reporter gene encoding **Lucia luciferase**, a secreted luciferase. Thus, activation of the IFN pathway can be assessed by measuring the levels of Lucia luciferase, which are directly proportional to the amount of type I IFNs produced and readily monitored using the **QUANTI-Luc™ bioluminescent assay**.

The knockout status of each cell line is thoroughly tested as follows:

- *Biallelic deletion confirmed by PCR and DNA sequencing*
- *Reporter activity validated by functional assays*
- *Knockout stability guaranteed up to 20 passages*

RAW-Lucia™ ISG KO Cells

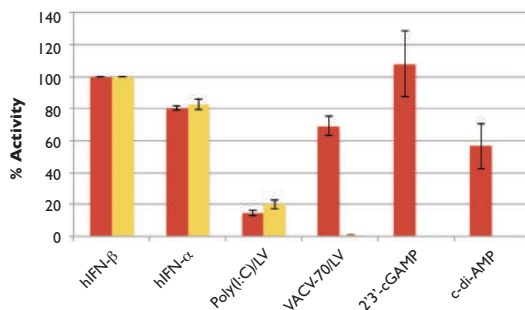
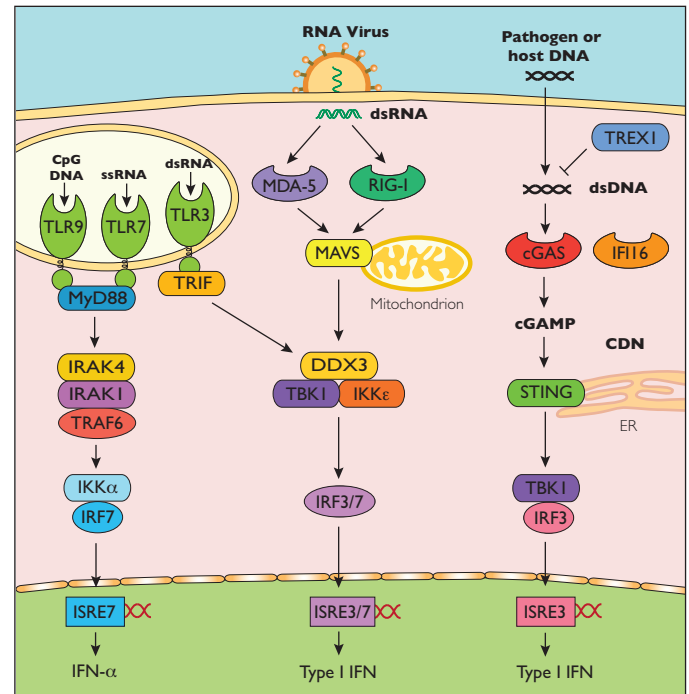
RAW-Lucia™ ISG-KO cells are a family of KO cell lines generated from RAW-Lucia™ ISG cells, a murine RAW 264.7 macrophage-derived cell line. They express the Lucia luciferase gene under control of the I-ISG54 promoter which comprises the IFN-inducible ISG54 promoter enhanced by a multimeric ISRE.

RAW-Lucia™ ISG-KO cells are resistant to Zeocin™. They are grown in DMEM medium, 2mM L-glutamine, 10% FBS supplemented with 200 µg/ml Zeocin™. They are provided in vials containing 3-7 × 10⁶ cells, and they come supplied with 10 mg Zeocin™, 50 mg Normocin™ and 1 pouch of QUANTI-Luc™ (see next page).

THPI-Dual™ KO Cells

THPI-Dual™ KO cells are a family of KO cell lines generated from THPI-Dual™ cells, a human THP-1 monocyte-derived cell line. They express the Lucia luciferase gene under the control of the I-ISG54 promoter. In addition, they coexpress the SEAP (secreted embryonic alkaline phosphatase) reporter gene driven by the IFN-β minimal promoter fused to five NF-κB binding sites. Accordingly, THPI-Dual™ KO cells enable simultaneous study of the IFN pathway, by monitoring Lucia luciferase activity, and the NF-κB pathway, by assessing SEAP activity.

THPI-Dual™ KO cells are resistant to blasticidin and Zeocin™. They are grown in RPMI medium, 2mM L-glutamine, 10% FBS supplemented with 10 µg/ml blasticidin and 100 µg/ml Zeocin™. They are provided in vials containing 3-7 × 10⁶ cells and supplied with 1 mg blasticidin, 10 mg Zeocin™, 50 mg Normocin™, 1 pouch of QUANTI-Luc™ and 1 pouch of QUANTI-Blue™ (see next page).



IFN Response of THPI-Dual™ ISG-KO-STING and THPI-Dual™ ISG cells to various stimuli. Cells were incubated with poly(I:C) LMW/LyoVec™ (100 ng/ml), VACV-70/LyoVec™ (1 µg/ml), 2'3'-cGAMP (3 µg/ml), and c-di-AMP (10 µg/ml). Human IFN-α and IFN-β (1 × 10⁴ U/ml) serve as positive controls. After 24h incubation, IRF activation was determined by measuring the relative light units (RLUs) in a luminometer using QUANTI-Luc™. The IRF induction of each ligand is expressed relative to that of hIFN-β at 1 × 10⁴ U/ml (taken as 100%).

PRODUCT	CAT. CODE
RAW-Lucia ISG KO Cells (3-7 × 10⁶ cells)	
RAW-Lucia™ ISG-KO-cGAS Cells	rawl-kocgas
RAW-Lucia™ ISG-KO-IFI16 Cells	rawl-koif16
RAW-Lucia™ ISG-KO-IRF3 Cells	rawl-koirf3
RAW-Lucia™ ISG-KO-IRF7 Cells NEW	rawl-koirf7
RAW-Lucia™ ISG-KO-MDA5 Cells NEW	rawl-komda5
RAW-Lucia™ ISG-KO-RIG-I Cells	rawl-korigi
RAW-Lucia™ ISG-KO-STING Cells	rawl-kostg
RAW-Lucia™ ISG-KO-TBK1 Cells	rawl-kotbk
RAW-Lucia™ ISG-KO-TREX1 Cells	rawl-kotrex
RAW-Lucia™ ISG-KO-TRIF Cells NEW	rawl-kotrif
THPI-Dual KO Cells (3-7 × 10⁶ cells)	
THPI-Dual™ KO-MyD Cells	thpd-komyd
THPI-Dual™ KO-STING Cells NEW	thpd-kostg
THPI-Dual™ KO-TREX1 Cells NEW	thpd-kotrex
THPI-Dual™ KO-IFI16 Cells NEW	thpd-koif16
Parental Cell Lines (3-7 × 10⁶ cells)	
RAW-Lucia™ ISG Cells	rawl-isg
THPI-Dual™ Cells	thpd-nfis

Reporter Detection Reagents

One-step reagents for detection of SEAP or Luciferase

Secreted embryonic alkaline phosphatase (SEAP) and luciferase are two reporter proteins widely used to study promoter activity or gene expression. SEAP is a truncated form of human placental alkaline phosphatase that hydrolyzes a variety of substrates. It is secreted into the cell culture medium and detected by testing aliquots of medium, leaving cells intact for further experimentation. Luciferase is a family of proteins of different origins that utilize the bioluminescent substrates luciferin or coelenterazine to produce light. InvivoGen offers Lucia luciferase, a coelenterazine-utilizing luciferase that has the advantage of being secreted and of producing a stabilized light signal. To facilitate detection of SEAP or of Lucia-luciferase and other coelenterazine-utilizing luciferases (e.g. Renilla luciferase and Gaussia luciferase), InvivoGen has developed one-step reagents that are easy to use, sensitive, reliable and economical.

SEAP DETECTION

QUANTI-Blue™ - Detection & quantification of SEAP

QUANTI-Blue™ is a detection reagent developed to determine the levels of SEAP in biological samples. It offers many advantages over the conventional SEAP Reporter Assay Kit based on the pNPP substrate, including ease-of-use, short hands-on-time and visual readout (color change from pink to purple/blue). The same cell cultures can be repeatedly sampled for kinetics studies or further experimentation. SEAP activity can be detected as early as 15 min after incubation of the samples in QUANTI-Blue™. SEAP activity can be assessed qualitatively with the naked eye or quantitatively with a spectrophotometer by measuring the absorbance at 620-655 nm.

HEK-Blue Detection™ - Real-time detection of SEAP

HEK-Blue™ Detection is a cell culture medium that detects SEAP as it is secreted by the cells. It contains all the nutrients necessary for cell growth as well as a SEAP-specific colorimetric substrate. Hydrolysis of this substrate by SEAP produces a purple/blue color that can be easily detected with the naked eye or measured with a spectrophotometer.

LUCIFERASE DETECTION

QUANTI-Luc™ - Quantification of luciferases

QUANTI-Luc™ is a lyophilized assay reagent containing all the components required to quantitatively measure the activity of Lucia luciferase and other coelenterazine-utilizing luciferases. It contains coelenterazine, the substrate for the luciferase reaction, which generates a light signal that can be quantified using a luminometer and expressed in relative light units (RLU). Thus, the signal produced correlates to the amount of luciferase protein expressed, indicating promoter activity in the reporter assay.

PRODUCT	QUANTITY	CAT. CODE
SEAP Detection Reagents		
HEK-Blue™ Detection	5 pouches (5 x 50 ml)	hb-det2
	10 pouches (10 x 50 ml)	hb-det3
QUANTI-Blue™	5 pouches (5 x 100 ml)	rep-qb1
	10 pouches (10 x 100 ml)	rep-qb2
Luciferase Detection Reagent		
QUANTI-Luc™	2 pouches (2 x 25 ml)	rep-qlc1
	5 pouches (5 x 25 ml)	rep-qlc2

Endotoxin-free Selective Antibiotics

Functionally validated antibiotics

InvivoGen offers a range of endotoxin-free selective antibiotics to ensure clean selection of transfected mammalian cells. These antibiotics are functionally validated through rigorous physico-chemical, microbiological and cellular testing. They exhibit proven long-term stability to mammalian cells, with no cytotoxicity confirmed through four passages.

The importance of endotoxin-free

Endotoxins, also known as lipopolysaccharides (LPS), are strongly immunomodulatory substances that derive from the outer membrane of Gram-negative bacteria. Contamination by endotoxins can functionally alter or even kill mammalian cells, with major consequences for cell signaling pathways and gene transfection. InvivoGen ensures that its selective antibiotics are endotoxin-free by testing them with a chromogenic Limulus amoebocyte lysate (LAL) assay. This step is critical for preventing contamination of transfected cells during treatment with selective antibiotics.

PRODUCT	QUANTITY	CAT. CODE
Blasticidin	100 mg (10 x 1 ml)	ant-bl-1
G418 (Geneticin)	1 g (10 x 1 ml)	ant-gn-1
Hygromycin B Gold	1 g (10 x 1 ml)	ant-hg-1
Puromycin	100 mg (10 x 1 ml)	ant-pr-1
Zeocin	1 g (10 x 1 ml)	ant-zn-1

