Drugs of the Future 2008, 33(5): 396-401

CCC: 0377-8282/2008
DOI: 10.1358/dof.2008.033.05.1201624

INGN-241

Cancer Gene Therapy

Ad-mda7
Ad-mda7/IL-24

Adenoviral vector carrying the melanoma differentiation-associated gene 7 (*MDA-7*)

EN: 296924

Abstract

The profound toxic effects of conventional cancer therapies such as chemo- and radiotherapy and the nonspecific side effects of small-molecule inhibitors of signaling proteins mandate more effective and preferably nontoxic approaches for treating cancer. An ideal cancer therapeutic should specifically and selectively target not only neoplastic cells but also a tumor's blood supply, and should effectively eradicate both primary and metastatic tumors with equal efficiency. Although this combination of properties in a single or even multiple agents seems untenable, melanoma differentiation-associated gene 7/interleukin-24 (*MDA-7/IL24*) exhibits all of these traits, thus attaining the unique status of a potential 'magic bullet' for cancer. This unique IL-10 family member cytokine induces apoptosis only in cancer cells without harming normal cells, inhibits tumor angiogenesis, stimulates an antitumor immune response, synergizes with radiation, chemotherapy, small molecules and monoclonal antibodies, and thereby inhibits both primary and metastatic tumors by eliciting a profound 'bystander' antitumor effect. A phase I clinical trial has been successfully completed using a replication-incompetent adenovirus expressing *MDA-7/IL24* (INGN-241), demonstrating safety, lack of toxicity and significant objective clinical responses. Thus, *MDA-7/IL24* therapy might promote the elimination of primary and distant tumors, resulting in prolonged survival of patients and establishing a 'cure'.

Background

What properties would in principle comprise a perfect cancer gene therapeutic? These would include: 1) selective killing of cancer cells while sparing normal cells from harm; 2) induction of a 'bystander' anticancer effect; 3) facilitating the destruction of both primary and metastatic tumors; and 4) evoking additional multipronged antitumor effects, thereby maximizing elimination of neoplastic cells (1). Melanoma differentiation-associated gene 7 (*MDA-7*)/interleukin-24 (*IL24*) exhibits all of these characteristics and more. This cytokine induces apoptosis specifically in various cancer cells, without exerting injurious effects to their normal counterparts, reduces angiogenesis, stimulates a potent antitumor immune response, increases the sensitivity of cancer cells to radiation and other modalities of therapy, including chemotherapy and monoclonal antibody (mAb) therapy, and exhibits profound 'bystander' antitumor activity, destroying both primary and distant tumors in animal models (2-17).

Dedifferentiation is a hallmark of many neoplastic cells and reprogramming of differentiation, whereby cancer cells are reverted to a more differentiated normal phenotype, constitutes one modality of anticancer therapy (18). In human melanoma cells, exposure to interferon beta and the protein kinase C (PKC) activator mezerein induces terminal differentiation characterized by irreversible growth arrest and morphological, biochemical, antigenic and gene expression changes characteristic of the phenotype of normal melanocytes (19-21). Differential gene expression analysis identified MDA genes whose expression is induced during terminal differentiation, and *MDA-7* was cloned as a novel gene showing marked and sustained augmentation of expression upon treatment with interferon beta and mezerein (21-23). Sequence analysis, chromosomal localization and biochemical properties identified *MDA-7* as a secreted cytokine

Devanand Sarkar[1,2*+], Paul Dent[3], David T. Curiel[4], Paul B. Fisher[1,2,5*]. Departments of Pathology[1], Urology[2] and Neurosurgery[5], Herbert Irving Comprehensive Cancer Center, Columbia University, New York, NY 10032, USA; [3]Department of Biochemisty, Massey Cancer Center, Virginia Commonwealth University, Richmond, VA, USA; [4]Division of Human Gene Therapy, Gene Therapy Center, University of Alabama, Birmingham, AL 35294, USA. [+]Present address: Department of Human and Molecular Genetics, Massey Cancer Center, Virginia Commonwealth University, Richmond, VA, USA. *Correspondence: dsarkar@vcu.edu and pbf1@columbia.edu

belonging to the interleukin *IL10* gene family, along with *IL19, IL20, IL22* and *IL26*, and *MDA-7* was designated *IL24* (24, 25).

MDA-7/IL-24 is a 206-amino-acid, heavily glycosylated protein. Its expression is detected in human immune tissues, such as spleen, thymus, peripheral blood mononuclear cells (PBMCs) and normal melanocytes (25). This cytokine binds to its cognate receptor complexes, consisting of two sets of heterodimeric chains, IL-20R1 and IL-20R2 or IL-22R1 and IL-20R2, and activates signal transducer and activator of transcription 3 (STAT3) signaling (26, 27). MDA-7/IL-24 production is induced in PBMCs and monocytes by treatment with lipopolysaccharide (LPS), or in T cells, especially in $CD4^+$ naïve and memory cells, following activation by an anti-CD3 mAb (11, 28). Treatment of PBMCs with MDA-7/IL-24 protein induces proinflammatory cytokine production and MDA-7/IL-24 protein also plays a role in T2 lymphocyte differentiation (regulating humoral immunity) (29). Recent studies demonstrate a role for IL-24 in the proliferation of primary keratinocytes that express receptors for IL-24 and in the pathogenesis of psoriasis (30-32). IL-24, along with IL-19, IL-20 and IL-22, is induced in primary keratinocytes upon IL-1β treatment and its expression is detected in psoriatic skin (30, 31). IL-24 treatment resulted in acanthosis of reconstituted human epidermis, with induction of psoriasis-associated genes indicating its potential involvement in regulating skin biology (32).

Overexpression studies to elucidate the functions of *MDA-7/IL24* provided clues to its anticancer properties. Interestingly, forced expression of *MDA-7/IL24* at a supraphysiological level, such as via an adenoviral vector (Ad-*mda-7*), did not induce terminal differentiation in human melanoma cells, although there was a profound induction of cell death by apoptosis (33). This induction of apoptosis has been observed in almost all types of cancers, except for pancreatic cancer. The most intriguing observation is the absence of apoptosis induction in any form of normal cells, *i.e.*, epithelial cells, fibroblasts, melanocytes, mesenchymal cells and astrocytes, upon Ad-*mda-7* infection, indicating its possible utility as an anticancer gene therapeutic (2-5). Indeed, multiple groups have confirmed this cancer-selective apoptosis induction in diverse tumor indications, as well as a plethora of indirect antitumor properties for Ad-*mda-7*.

A replication-incompetent adenovirus expressing *MDA-7/IL24* (INGN-241, Ad-mda7) has been evaluated in a phase I clinical trial for multiple solid tumors and melanoma, demonstrating safety and significant objective clinical responses in patients (34, 35). A phase II study is in progress evaluating INGN-241 as monotherapy in patients with advanced melanoma (36) and it is in phase III evaluation for solid tumors in combination with radiotherapy.

Preclinical Pharmacology

The most enigmatic question is how *MDA-7/IL24* induces apoptosis differentially in cancer cells while sparing normal cells. The mechanism explaining this phenomenon remains to be elucidated, although certain traits are observed only in cancer cells and not in normal cells that might underlie this differential response. Another intriguing observation is that although the ultimate endpoint of *MDA-7/IL24* action is the induction of apoptosis, depending on the cell type and experimental context this cytokine activates different signal transduction pathways to achieve this objective. *MDA-7/IL24* induces cancer-selective apoptosis by an intracellular mode of action that does not require intact Janus kinase (JAK)/STAT signaling, a classical cytokine signaling cascade, or secretion of the molecule (37, 38). *MDA-7/IL24* localizes predominantly in the endoplasmic reticulum (ER)/Golgi compartment and induces an ER stress response also known as an 'unfolded protein response' (UPR) (39). In the ER, *MDA-7/IL24* interacts with the ER chaperone protein BiP/GRP78 (immunoglobulin heavy chain-binding protein/78 kDa glucose-regulated protein), which results in activation of p38 mitogen-activated protein kinase (MAPK) and subsequent induction of growth arrest and the DNA damage-inducible (*GADD*) family of genes (39, 40). Mutation in the BiP/GRP78-binding motifs in *MDA-7/IL24* abolishes p38 MAPK induction, *GADD* gene induction, as well as apoptosis (39). However, *MDA-7/IL24* interacts with BiP/GRP78 in both normal and cancer cells, although p38 MAPK activation and apoptosis induction are evident only in cancer cells, indicating the possible presence of a cancer-selective mediator of BiP/GRP78 downstream signaling. Identification of this downstream molecule might provide significant insight into the cancer selectivity of *MDA-7/IL24*. Additionally, levels of BiP/GRP78 have been shown to be higher in multiple cancer cells than in normal cells and the relative level of BiP/GRP78 might determine sensitivity to *MDA-7/IL24* (41, 42).

Ad-*mda-7* induces apoptosis of lung cancer cells via a direct interaction, and the involvement of upregulation of double-stranded RNA-activated protein kinase (PKR) and activation of c-Jun kinase (JNK) pathways in mediating Ad-*mda-7*-induced apoptosis has also been documented in the context of radiosensitization (10, 43). All these kinases, p38 MAPK, PKR and JNK, function downstream of the ER stress signaling pathway, suggesting a central role of ER stress induction as a potential mechanism of Ad-*mda-7* anticancer function. Ad-*mda-7* has been shown to inhibit β-catenin and phosphatidylinositol 3-kinase (PI3K) signaling pathways in lung cancer cells and to activate Fas-FasL signaling in ovarian cancer cells (44, 45). Ad-*mda-7* also generates reactive oxygen species (ROS) in the mitochondria, shifts the balance between pro- and antiapoptotic proteins and sets forth the cascade of intrinsic (mitochondrial) apoptotic signaling (46, 47). Forced overexpression of the antiapoptotic protein Bcl-2 or Bcl-X_L renders prostate cancer cells resistant to Ad-*mda-7*, which might be overcome by either ROS generators or radiation (48, 49). Multiple studies have demonstrated that the basal ROS levels in cancer cells are higher than in normal cells (50). As such, exogenous

agents that promote ROS production can overcome natural antioxidants much less efficiently in cancer cells as opposed to normal cells, thus inducing apoptosis and cell death. This is the rationale for using arsenic trioxide and other oxidative stress inducers to promote apoptosis selectively in cancer cells, and this phenomenon might also be the causal event for the cancer selectivity of *MDA-7/IL24*.

Among cancer cells, pancreatic carcinomas are conspicuous for their inherent resistance to Ad-*mda-7*-mediated apoptosis due to selective inhibition of the translation of *MDA-7/IL24* mRNA (51, 52). Activated mutations in the *KRAS* oncogene are observed in > 95% of pancreatic cancer patients and this process appears to occur early in the etiology of the disease. Inhibition of *KRAS* by either chemical or genetic approaches relieves *MDA-7/IL24* translational inhibition and makes both wild-type and mutant *KRAS*-containing pancreatic cancer cells sensitive to Ad-*mda-7* (52, 53). ROS generators also relieve this inhibition in a *KRAS*-independent manner in pancreatic carcinoma cells (47, 53). A single study by Introgen Therapeutics, however, demonstrated that Ad-mda7 as a single agent inhibited the growth of human pancreatic cancer cells by inhibiting Wnt/PI3K signaling (54). These discrepant findings might be explained by subtle differences in vector construction, one of the vectors having a β-globin polyadenylation signal and the other an SV40 polyadenylation signal (35, 55), as well as in doses of the viruses used in the studies.

In addition to its direct effect on cancer cells resulting in apoptosis, Ad-*mda-7* also decreases tumor growth by inhibiting angiogenesis. Ad-*mda-7* does not affect the growth of normal human vascular endothelial cells (HUVECs), but inhibits *in vitro* the differentiation of and tube formation by HUVECs and inhibits angiogenesis in nude mice tumor xenograft models (6, 7). Similarly, purified MDA-7/IL-24 protein inhibits HUVEC differentiation and migration, but not proliferation. Addition of blocking antibody to IL-22R1 protects HUVECs from MDA-7/IL-24 effects, indicating that a receptor interaction mediates this effect (7). Vascular endothelial growth factor (VEGF) expression is also inhibited by Ad-*mda-7* in lung and prostate cancer cells. In prostate cancer cells, inhibition of c-Src (cellular homologue of avian sarcoma viral oncogene) kinase activity by Ad-*mda-7* confers VEGF inhibition (56).

Ad-*mda-7* synergizes with radiation to induce apoptosis in various cancer cells. Its radiosensitizing properties have been demonstrated in glioblastoma multiforme, lung and ovarian cancers, as well as parental or Bcl-2- or Bcl-X_L-overexpressing prostate cancers (8-10, 15, 49). Activation of the JNK pathway plays a key role in this effect (10). In lung cancer cells, Ad-*mda-7* augments the therapeutic effects of epidermal growth factor (EGF) receptor inhibitors, such as gefitinib or erlotinib, bevacizumab, a humanized monoclonal antibody against VEGF, and geldanamycin, a chemotherapeutic (12, 13, 57). Ad-*mda-7* synergizes with trastuzumab, a monoclonal antibody against HER2/NEU, in breast cancer cells (14). Interestingly, nonsteroidal antiinflammatory drugs such as sulindac induce apoptosis in cancer cells by promoting *MDA-7/IL24* expression, and also augment Ad-*mda-7*-induced apoptosis by enhancing *MDA-7/IL24* mRNA stability (58, 59).

The receptor-dependent apoptosis-inducing properties underlie the 'bystander effect' of secreted MDA-7/IL-24 protein. Transfection of only about 8% of pancreatic cancer cells significantly inhibited the growth of established tumors in nude mice (50). MDA-7/IL-24 protein secreted from the normal cells effectively inhibited the growth of cocultivated cancer cells (16). Subcutaneous xenografts from human breast cancer and resistant prostate cancer cells were established on both the right and left flanks of athymic nude mice and only the tumors on the left side were injected with Ad-*mda-7* (17, 60). Of therapeutic relevance, Ad-*mda-7* not only inhibited the growth of the left-sided tumors that were injected, but also significantly inhibited the right-sided noninjected tumors. Tumors were established in mouse spleen using mouse hepatoma cells that metastasized to the liver. Electroporation-mediated delivery of plasmids expressing mouse *mda-7/IL24* to the quadriceps muscle resulted in a significant increase in survival and a reduction of metastatic foci in the liver, although it did not have any profound effect on the primary tumor established in the spleen (61).

Indirect evidence that *MDA-7/IL24* stimulates the immune system by inducing the production of proinflammatory cytokines has been observed in *in vitro* and clinical studies (11, 29, 34, 35). A recent study employing immunocompetent animals further extended these observations (62). Murine fibrosarcoma UV2237m cells infected with Ad-*mda-7* did not grow in syngeneic immunocompetent C3H mice. These tumor-free C3H mice, when challenged with parental tumor cells, experienced no tumor growth, suggesting induction of systemic immunity. Splenocytes prepared from vaccinated C3H mice demonstrated higher proliferative activity and produced elevated levels of Th1 cytokines compared with those from control mice, and demonstrated a significant increase in the $CD3^+CD8^+$ cell population. Thus, Ad-*mda-7* treatment of syngeneic tumors promotes immune activation, leading to anticancer immunity.

Clinical Studies

The *in vitro* and *in vivo* animal studies demonstrating the profound but nontoxic anticancer efficacy of Ad-*mda-7* paved the way for its translation into the clinic. Presently, only one phase I clinical trial has been carried out using INGN-241, a replication-incompetent adenovirus expressing *MDA-7/IL24* developed by Introgen Therapeutics, in 28 patients with multiple solid tumors administered the therapy by intratumoral injection (34, 35). INGN-241 was created on an Ad5 backbone with E1A and partial E3 region deletions using a cytomegalovirus (CMV) immediate early promoter to drive *MDA-7/IL24* expression, followed by an SV40 polyadenylation signal. In all patients receiving single or repeated intratumoral

injections of 2 x 10^{10}-2 x 10^{12} viral particles (vp), adverse events were mild, consisting of injection-site pain and fever (35). Patients receiving single injections did not show any clinical response. Eight patients received twice-weekly injections for 3 weeks (on a 28-day cycle for 1 or more cycles) and 5 patients completed at least 1 cycle of treatment. Two of these patients demonstrated a clinically significant response to INGN-241, consisting of at least partial regression of the injected lesion. The most dramatic response was observed in a 64-year-old female with widely metastatic melanoma and more than 10 distinct lesions. A lesion in the supraclavicular node (2 x 2 cm) was injected with INGN-241 6 times, which resulted in gradual regression over the next 2 weeks and no clinical evidence of disease. A lesion on the dorsum of the right hand (1.8 x 2.3 cm) was treated next and with 5 injections there was an 84% reduction in lesion area, with microscopic lymphoplasmacytic infiltrations and extensive coagulative necrosis. A third lesion in the anterior right thigh (3.5 x 3 cm) showed a 35% reduction. This patient survived for 773 days after initiation of INGN-241 treatment and an additional melanoma patient exhibited a partial response (33% decrease). Five patients survived more than 2 years, and 4 were still alive at 1,643, 1,550, 1,267 and 1,261 days.

The immunostimulatory activity of *MDA-7/IL24* was demonstrated in the clinical trial (34). Transient increases in circulating cytokines, such as IL-6, IL-10 and tumor necrosis factor α (TNF-α), were observed with INGN-241 injection. The majority of patients also showed a marked increase in $CD3^+$ and $CD8^+$ T cells at day 15 following injection, suggesting that INGN-241 may be associated with a Th1 response. These initial clinical results revealed that INGN-241 is well tolerated when administered via intratumoral injection and repeated dosing with 2 x 10^{12} vp/injection could be utilized in subsequent clinical investigations via intratumoral injection.

Conclusions

With the initial success of a phase I clinical trial in multiple solid tumors, it is essential that INGN-241 be evaluated further in phase II/III trials for the treatment of cancer patients. Conditionally replication-competent bipartite adenoviruses that replicate only in cancer cells and simultaneously express *MDA-7/IL24* have shown a profound effect in eliminating primary and distant tumors derived from human breast, colorectal and resistant prostate cancer cells in nude mouse models (17, 60, 63). Additionally, delivery of *MDA-7/IL24* using adeno-associated virus (AAV) has also demonstrated significant efficacy (64). These findings further stress that INGN-241 and modified and improved versions have profound potential for cancer treatment in the future.

Acknowledgements

The present studies were supported in part by National Institutes of Health grants R01 CA097318 and P01 CA104177, and the Samuel Waxman Cancer Research Foundation. PBF is the Michael and Stella Chernow Urological Cancer Research Scientist and a SWCRF Investigator.

Source

Introgen Therapeutics, Inc. (US).

References

1. Buchsbaum, D.J., Curiel, D.T. *Gene therapy for the treatment of cancer*. Cancer Biother Radiopharm 2001, 16(4): 275-88.

2. Sarkar, D., Lebedeva, I.V., Gupta, P. et al. *Melanoma differentiation associated gene-7 (mda-7)/IL-24: A 'magic bullet' for cancer therapy?* Expert Opin Biol Ther 2007, 7(5): 577-86.

3. Fisher, P.B., Sarkar, D., Lebedeva, I.V. et al. *Melanoma differentiation associated gene-7/interleukin-24 (mda-7/IL-24): Novel gene therapeutic for metastatic melanoma*. Toxicol Appl Pharmacol 2007, 224(3): 300-7.

4. Gupta, P., Su, Z.Z., Lebedeva, I.V. et al. *mda-7/IL-24: Multifunctional cancer-specific apoptosis-inducing cytokine*. Pharmacol Ther 2006, 111(3): 596-628.

5. Lebedeva, I.V., Sauane, M., Gopalkrishnan, R.V. et al. *mda-7/IL-24: Exploiting cancer's Achilles' heel*. Mol Ther 2005, 11(1): 4-18.

6. Nishikawa, T., Ramesh, R., Munshi, A., Chada, S., Meyn, R.E. *Adenovirus-mediated mda-7 (IL24) gene therapy suppresses angiogenesis and sensitizes NSCLC xenograft tumors to radiation*. Mol Ther 2004, 9(6): 818-28.

7. Ramesh, R., Mhashilkar, A.M., Tanaka, F. et al. *Melanoma differentiation-associated gene 7/interleukin (IL)-24 is a novel ligand that regulates angiogenesis via the IL-22 receptor*. Cancer Res 2003, 63(16): 5105-13.

8. Kawabe, S., Nishikawa, T., Munshi, A., Roth, J.A., Chada, S., Meyn, R.E. *Adenovirus-mediated mda-7 gene expression radiosensitizes non-small cell lung cancer cells via TP53-independent mechanisms*. Mol Ther 2002, 6(5): 637-44.

9. Su, Z.Z., Lebedeva, I.V., Sarkar, D. et al. *Melanoma differentiation associated gene-7, mda-7/IL-24, selectively induces growth suppression, apoptosis and radiosensitization in malignant gliomas in a p53-independent manner*. Oncogene 2003, 22(8): 1164-80.

10. Yacoub, A., Mitchell, C., Lebedeva, I.V. et al. *mda-7 (IL-24) inhibits growth and enhances radiosensitivity of glioma cells in vitro via JNK signaling*. Cancer Biol Ther 2003, 2(4): 347-53.

11. Caudell, E.G., Mumm, J.B., Poindexter, N. et al. *The protein product of the tumor suppressor gene, melanoma differentiation-associated gene 7, exhibits immunostimulatory activity and is designated IL-24*. J Immunol 2002, 168(12): 6041-6.

12. Pataer, A., Bocangel, D., Chada, S., Roth, J.A., Hunt, K.K., Swisher, S.G. *Enhancement of adenoviral MDA-7-mediated cell killing in human lung cancer cells by geldanamycin and its 17-allyl-amino-17-demethoxy analogue*. Cancer Gene Ther 2007, 14(1): 12-8.

13. Emdad, L., Lebedeva, I.V., Su, Z.Z., Gupta, P., Sarkar, D., Settleman, J., Fisher, P.B. *Combinatorial treatment of non-small-*

cell lung cancers with gefitinib and Ad.mda-7 enhances apoptosis-induction and reverses resistance to a single therapy: Implications for clinical use. J Cell Physiol 2007, 210(2): 549-59.

14. Bocangel, D., Zheng, M., Mhashilkar, A., Liu, Y., Ramesh, R., Hunt, K.K., Chada, S. *Combinatorial synergy induced by adenoviral-mediated mda-7 and Herceptin in Her-2+ breast cancer cells*. Cancer Gene Ther 2006, 13(10): 958-68.

15. Emdad, L., Sarkar, D., Lebedeva, I.V. et al. *Ionizing radiation enhances adenoviral vector expressing mda-7/IL-24-mediated apoptosis in human ovarian cancer*. J Cell Physiol 2006, 208(2): 298-306.

16. Su, Z.Z., Emdad, L., Sauane, M. et al. *Unique aspects of mda-7/IL-24 antitumor bystander activity: Establishing a role for secretion of MDA-7/IL-24 protein by normal cells*. Oncogene 2005, 24(51): 7552-66.

17. Sarkar, D., Su, Z.Z., Vozhilla, N., Park, E.S., Gupta, P., Fisher, P.B. *Dual cancer-specific targeting strategy cures primary and distant breast carcinomas in nude mice*. Proc Natl Acad Sci USA 2005, 102(39): 14034-9.

18. Leszczyniecka, M., Roberts, T., Dent, P., Grant, S., Fisher, P.B. *Differentiation therapy of human cancer: Basic science and clinical applications*. Pharmacol Ther 2001, 90(23): 105-56.

19. Fisher, P.B., Grant, S. *Effects of interferon on differentiation of normal and tumor cells*. Pharmacol Ther 1985, 27(2): 143-66.

20. Guarini, L., Graham, G.M., Jiang, H. et al. *Modulation of the antigenic phenotype of human melanoma cells by differentiation-inducing and growth-suppressing agents*. Pigment Cell Res 1992, Suppl. 2: 123-31.

21. Jiang, H., Lin, J., Fisher, P.B. *A molecular definition of terminal cell differentiation in human melanoma cells*. Mol Cell Differ 1994, 2: 221-39.

22. Jiang, H., Su, Z.Z., Boyd, J., Fisher, P.B. *Gene expression changes associated with reversible growth suppression and the induction of terminal differentiation in human melanoma cells*. Mol Cell Differ 1993, 1: 41-66.

23. Jiang, H., Lin, J.J., Su, Z.Z., Goldstein, N.I., Fisher, P.B. *Subtraction hybridization identifies a novel melanoma differentiation associated gene, mda-7, modulated during human melanoma differentiation, growth and progression*. Oncogene 1995, 11: 2477-86.

24. Pestka, S., Krause, C.D., Sarkar, D., Walter, M.R., Shi, Y., Fisher, P.B. *Interleukin-10 and related cytokines and receptors*. Annu Rev Immunol 2004, 22: 929-79.

25. Huang, E.Y., Madireddi, M.T., Gopalkrishnan, R.V. et al. *Genomic structure, chromosomal localization and expression profile of a novel melanoma differentiation associated (mda-7) gene with cancer specific growth suppressing and apoptosis inducing properties*. Oncogene 2001, 20(48): 7051-63.

26. Wang, M., Tan, Z., Zhang, R., Kotenko, S.V., Liang, P. *Interleukin 24 (MDA-7/MOB-5) signals through two heterodimeric receptors, IL-22R1/IL-20R2 and IL-20R1/IL-20R2*. J Biol Chem 2002, 277(9): 7341-7.

27. Dumoutier, L., Leemans, C., Lejeune, D., Kotenko, S.V., Renauld, J.C. *Cutting edge: STAT activation by IL-19, IL-20 and mda-7 through IL-20 receptor complexes of two types*. J Immunol 2001, 167(7): 3545-9.

28. Wolk, K., Kunz, S., Asadullah, K., Sabat, R. *Cutting edge: Immune cells as sources and targets of the IL-10 family members?* J Immunol 2002, 168(11): 5397-402.

29. Poindexter, N.J., Walch, E.T., Chada, S., Grimm, E.A. *Cytokine induction of interleukin-24 in human peripheral blood mononuclear cells*. J Leukoc Biol 2005, 78(3): 745-52.

30. Boniface, K., Lecron, J.C., Bernard, F.X., Dagregorio, G., Guillet, G., Nau, F., Morel, F. *Keratinocytes as targets for interleukin-10-related cytokines: A putative role in the pathogenesis of psoriasis*. Eur Cytokine Netw 2005, 16(4): 309-19.

31. Kunz, S., Wolk, K., Witte, E. et al. *Interleukin (IL)-19, IL-20 and IL-24 are produced by and act on keratinocytes and are distinct from classical ILs*. Exp Dermatol 2006, 15(12): 991-1004.

32. Sa, S.M., Valdez, P.A., Wu, J. et al. *The effects of IL-20 subfamily cytokines on reconstituted human epidermis suggest potential roles in cutaneous innate defense and pathogenic adaptive immunity in psoriasis*. J Immunol 2007, 178(4): 2229-40.

33. Jiang, H., Su, Z.Z., Lin, J.J. et al. *The melanoma differentiation associated gene mda-7 suppresses cancer cell growth*. Proc Natl Acad Sci USA 1996, 93(17): 9160-5.

34. Tong, A.W., Nemunaitis, J., Su, D. et al. *Intratumoral injection of INGN 241, a nonreplicating adenovector expressing the melanoma-differentiation associated gene-7 (mda-7/IL24): Biologic outcome in advanced cancer patients*. Mol Ther 2005, 11(1): 160-72.

35. Cunningham, C.C., Chada, S., Merritt, J.A. et al. *Clinical and local biological effects of an intratumoral injection of mda-7 (IL24; INGN 241) in patients with advanced carcinoma: A phase I study*. Mol Ther 2005, 11(1): 149-59.

36. *Safety and efficacy of INGN 241 gene therapy in patients with in transit melanoma (NCT00116363)*. ClinicalTrials.gov Web site, April 19, 2008.

37. Sauane, M., Gopalkrishnan, R.V., Lebedeva, I.V. et al. *Mda-7/IL-24 induces apoptosis of diverse cancer cell lines through JAK/STAT-independent pathways*. J Cell Physiol 2003, 196(2): 334-45.

38. Sauane, M., Lebedeva, I.V., Su, Z.Z. et al. *Melanoma differentiation associated gene-7/interleukin-24 promotes tumor cell-specific apoptosis through both secretory and nonsecretory pathways*. Cancer Res 2004, 64(9): 2988-93.

39. Gupta, P., Walter, M.R., Su, Z.Z. et al. *BiP/GRP78 is an intracellular target for MDA-7/IL-24 induction of cancer-specific apoptosis*. Cancer Res 2006, 66(16): 8182-91.

40. Sarkar, D., Su, Z.Z., Lebedeva, I.V. et al. *mda-7 (IL-24) mediates selective apoptosis in human melanoma cells by inducing the coordinated overexpression of the GADD family of genes by means of p38 MAPK*. Proc Natl Acad Sci USA 2002, 99(15): 10054-9.

41. Arap, M.A., Lahdenranta, J., Mintz, P.J., Hajitou, A., Sarkis, A.S., Arap, W., Pasqualini, R. *Cell surface expression of the stress response chaperone GRP78 enables tumor targeting by circulating ligands*. Cancer Cell 2004, 6(3): 275-84.

42. Lee, E., Nichols, P., Spicer, D., Groshen, S., Yu, M.C., Lee, A.S. *GRP78 as a novel predictor of responsiveness to chemotherapy in breast cancer*. Cancer Res 2006, 66(6): 7849-53.

43. Pataer, A., Vorburger, S.A., Barber, G.N. et al. *Adenoviral transfer of the melanoma differentiation-associated gene 7*

(mda7) induces apoptosis of lung cancer cells via up-regulation of the double-stranded RNA-dependent protein kinase (PKR). Cancer Res 2002, 62(8): 2239-43.

44. Mhashilkar, A.M., Stewart, A.L., Sieger, K. et al. *MDA-7 negatively regulates the beta-catenin and PI3K signaling pathways in breast and lung tumor cells*. Mol Ther 2003, 8(2): 207-19.

45. Gopalan, B., Litvak, A., Sharma, S. et al. *Activation of the Fas-FasL signaling pathway by MDA-7/IL-24 kills human ovarian cancer cells*. Cancer Res 2005, 65(8): 3017-24.

46. Lebedeva, I.V., Su, Z.Z., Sarkar, D. et al. *Melanoma differentiation associated gene-7, mda-7/interleukin-24, induces apoptosis in prostate cancer cells by promoting mitochondrial dysfunction and inducing reactive oxygen species*. Cancer Res 2003, 63(23): 8138-44.

47. Lebedeva, I.V., Su, Z.Z., Sarkar, D. et al. *Induction of reactive oxygen species renders mutant and wild-type K-ras pancreatic carcinoma cells susceptible to Ad.mda-7-induced apoptosis*. Oncogene 2005, 24(4): 585-96.

48. Lebedeva, I.V., Sarkar, D., Su, Z.Z. et al. *Bcl-2 and Bcl-xL differentially protect human prostate cancer cells from induction of apoptosis by melanoma differentiation associated gene-7, mda-7/IL-24*. Oncogene 2003, 22(54): 8758-73.

49. Su, Z.Z., Lebedeva, I.V., Sarkar, D. et al. *Ionizing radiation enhances therapeutic activity of mda-7/IL-24: Overcoming radiation- and mda-7/IL-24-resistance in prostate cancer cells overexpressing the antiapoptotic proteins bcl-xL or bcl-2*. Oncogene 2006, 25(16): 2339-48.

50. Pelicano, H., Carney, D., Huang, P. *ROS stress in cancer cells and therapeutic implications*. Drug Resist Updat 2004, 7(2): 97-110.

51. Su, Z.Z., Lebedeva, I.V., Gopalkrishnan, R.V. et al. *A combinatorial approach for selectively inducing programmed cell death in human pancreatic cancer cells*. Proc Natl Acad Sci USA 2001, 98(18): 10332-7.

52. Lebedeva, I.V., Sarkar, D., Su, Z.Z. et al. *Molecular target-based therapy of pancreatic cancer*. Cancer Res 2006, 6684): 2403-13.

53. Lebedeva, I.V., Washington, I., Sarkar, D. et al. *Strategy for reversing resistance to a single anticancer agent in human prostate and pancreatic carcinomas*. Proc Natl Acad Sci USA 2007, 104(9): 3484-9.

54. Chada, S., Bocangel, D., Ramesh, R., Grimm, E.A., Mumm, J.B., Mhashilkar, A.M., Zheng, M. *mda-7/IL24 kills pancreatic cancer cells by inhibition of the Wnt/PI3K signaling pathways: Identification of IL-20 receptor-mediated bystander activity against pancreatic cancer*. Mol Ther 2005, 11(5): 724-33.

55. Lebedeva, I.V., Su, Z.Z., Chang, Y., Kitada, S., Reed, J.C., Fisher, P.B. *The cancer growth suppressing gene mda-7 induces apoptosis selectively in human melanoma cells*. Oncogene 2002, 21(5): 708-18.

56. Inoue, S., Branch, C.D., Gallick, G.E., Chada, S., Ramesh, R. *Inhibition of Src kinase activity by Ad-mda7 suppresses vascular endothelial growth factor expression in prostate carcinoma cells*. Mol Ther 2005, 12(4): 707-15.

57. Inoue, S., Hartman, A., Branch, C.D. et al. *mda-7 In combination with bevacizumab treatment produces a synergistic and complete inhibitory effect on lung tumor xenograft*. Mol Ther 2007, 15(2): 287-94.

58. Zerbini, L.F., Czibere, A., Wang, Y. et al. *A novel pathway involving melanoma differentiation associated gene-7/interleukin-24 mediates nonsteroidal anti-inflammatory drug-induced apoptosis and growth arrest of cancer cells*. Cancer Res 2006, 66(24): 11922-31.

59. Oida, Y., Gopalan, B., Miyahara, R. et al. *Sulindac enhances adenoviral vector expressing mda-7/IL-24-mediated apoptosis in human lung cancer*. Mol Cancer Ther 2005, 4(2): 291-304.

60. Sarkar, D., Lebedeva, I.V., Su, Z.Z. et al. *Eradication of therapy-resistant human prostate tumors using a cancer terminator virus*. Cancer Res 2007, 67(11): 5434-42.

61. Chen, W.Y., Cheng, Y.T., Lei, H.Y., Chang, C.P., Wang, C.W., Chang, M.S. *IL-24 inhibits the growth of hepatoma cells in vivo*. Genes Immun 2005, 6(6): 493-9.

62. Miyahara, R., Banerjee, S., Kawano, K. et al. *Melanoma differentiation-associated gene-7 (mda-7)/interleukin (IL)-24 induces anticancer immunity in a syngeneic murine model*. Cancer Gene Ther 2006, 13(8): 753-61.

63. Zhao, L., Gu, J., Dong, A. et al. *Potent antitumor activity of oncolytic adenovirus expressing mda-7/IL-24 for colorectal cancer*. Hum Gene Ther 2005, 16(7): 845-58.

64. Tahara, I., Miyake, K., Hanawa, H. et al. *Systemic cancer gene therapy using adeno-associated virus type 1 vector expressing MDA-7/IL24*. Mol Ther 2007, 15(10): 1805-11.