Editorial

Targeting colorectal cancer (stem-like) cells using LGR5 directed antibody drug conjugates

Daniela Hirsch[1], Thomas Ried[2]

[1]Institute of Pathology, University Medical Center Mannheim, Heidelberg University, Mannheim, Germany; [2]Genetics Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

Correspondence to: Thomas Ried, MD. Center for Cancer Research, National Cancer Institute, Building 50, Room 1408, Bethesda, MD 20892-8010, USA. Email: riedt@mail.nih.gov.

Provenance: This is a Guest Editorial commissioned by Section Editor Junhong Wang, MD, PhD (Department of Geriatric Medicine, The first affiliated hospital of Nanjing Medical University, Nanjing, China).

Comment on: Junttila MR, Mao W, Wang X, *et al*. Targeting LGR5+ cells with an antibody-drug conjugate for the treatment of colon cancer. Sci Transl Med 2015;7:314ra186.

Submitted Oct 24, 2016. Accepted for publication Oct 29, 2016.
doi: 10.21037/atm.2016.11.78
View this article at: http://dx.doi.org/10.21037/atm.2016.11.78

Tumors are comprised of phenotypically and genotypically heterogeneous cell populations. The stem cell hypothesis of cancer proposes that tumors are organized hierarchically, in analogy to many normal organs and tissues that are renewed and maintained by adult tissue specific stem cells; in other words, a subpopulation of tumor cells possesses stem cell-like capabilities, hence being able to self-renew and to generate the diverse cells that comprise the bulk of the tumor. It is thought that so-called 'cancer stem cells' are involved in tumor relapse, metastasis and therapeutic resistance. Consequently, pursuing stem cells as therapeutic targets represents an interesting approach and might help to overcome some of the frustrations associated with current cancer treatment regimens. However, most cancer stem cell markers, often cell surface molecules, are not restricted to cancer cells but are also expressed in normal stem cells, making the selective targeting of cancer stem cells without harming normal tissue and organ function a challenge.

In the manuscript entitled "Targeting LGR5+ cells with an antibody-drug conjugate for the treatment of colon cancer" and published in *Science Translational Medicine* (1), Junttila and colleagues report an interesting approach using antibody drug conjugates (ADCs) to target cells expressing the putative colorectal cancer stem cell marker leucine-rich-repeat-containing G-protein-coupled receptor 5 (LGR5).

ADCs are composed of a cytotoxic drug linked to an antibody that recognizes a particular cell surface antigen and thus represent one way to selectively target a certain cell population (2). In simplified terms, ADCs bind to the target antigen, are internalized via endocytosis and thus ideally deliver the cytotoxic drug selectively to target antigen expressing cells. Targets of ADCs are either tumor-specific antigens that have no expression on normal cells or, as for the most part in practice, tumor-associated antigens that have restricted expression on normal cells. In general, target antigens of ADCs should be highly expressed on tumor cells with limited to no expression on normal cells.

The target antigen chosen by Junttila and colleagues, LGR5, is interesting as it has been reported to mark putative cancer stem cells in several tumor entities including colorectal cancer (3). Furthermore, it is overexpressed in various, predominantly gastrointestinal cancers. However, LGR5 expression is not restricted to cancer cells. Briefly, LGR5 is a G-protein-coupled, seven-transmembrane-domain receptor that acts as the receptor for the Wnt agonist R-spondin (4,5) and marks adult stem cells in various tissues and organs including stomach, small intestine, colon, hair follicles, ovary/tubal epithelia and kidney as shown by *in vivo* lineage tracing (6-10). *In vivo* studies further revealed that Lgr5 positive intestinal stem cells, unlike more differentiated cells, can serve as the cell-of-origin of mouse intestinal epithelial tumors (11) and lineage tracing in *Lgr5*$^{EGFP\text{-}IRES\text{-}CreERT2}$/*Apc*$^{fl/fl}$/*R26R-Confetti* mice provides direct evidence for a stem cell activity of

Lgr5 positive cells in intestinal adenomas (12). Interestingly, two stem cell pools exist in the intestine: the rapidly cycling crypt base columnar cells marked by Lgr5 and the rather quiescent/slowly cycling +4 cells marked by Bmi1, Hopx, Lrig1 and/or mTert (7,13-16). As shown by elimination of Lgr5 positive intestinal stem cells via diphtheria toxin, Lgr5 positive intestinal stem cells are dispensable for normal intestinal homeostasis under physiological conditions and +4 cells can compensate for the elimination of this population (17). In contrast, when intestinal epithelium has been injured such as post-radiation, Lgr5 positive cells proved to be indispensable for intestinal regeneration (18). Notably, Lgr5 depleted animals are not viable long-term due to reported liver complications (17). *In vitro* and *in vivo* data point to a stem cell function of Lgr5 positive cells in the liver in the case of regeneration upon tissue damage (19). Given the responsibilities of LGR5 positive cells in the intestine and other tissues and organs, it is unclear whether toxicity mediated by ADC dependent destruction of LGR5 positive cells will ultimately limit the use of LGR5 directed ADCs.

Junttila and colleagues have now generated a specific anti-LGR5 antibody which they subsequently conjugated to two cleavable linker-drugs with distinct mechanisms of action: the antimitotic microtubule inhibitor monomethyl auristatin E (MMAE) and the DNA damaging topoisomerase-inhibiting anthracycline PNU159682. This resulted in two anti-LGR5 specific ADCs, named anti-LGR5-MC-vc-PAB-MMAE (anti-LGR5-vc-MMAE) and anti-LGR5-NMS818.

In a first step, they applied their new anti-LGR5 antibody to study the expression of LGR5 in human colorectal carcinomas by immunohistochemistry. A heterogeneous expression of LGR5 was observed in colorectal carcinomas while in normal mucosa LGR5 expression was restricted to the bottom of normal intestinal crypts which is consistent with previous reports (7). LGR5 positivity was assigned when at least 10% of tumor cells showed expression of LGR5. While tissue microarray analysis of 143 colon cancers revealed a LGR5 positivity rate of 40% (57 of 143), a LGR5 positivity rate of 84% (16 of 19) was observed when using whole tissue sections. This is in line with previously published data showing a *LGR5* positivity rate of 74% (42 of 57) colorectal adenocarcinomas using mRNA *in situ* hybridization (20).

In vitro tests of their LGR5 specific ADCs, anti-LGR5-vc-MMAE and anti-LGR5-NMS818, on quiescent as well as actively dividing, normal and transformed cell lines (keratinocytes and SKBR3 human breast cancer cells) suggest a broader range of concentrations for cancer cell killing for anti-LGR5-NMS818, however, with a lower differential between its cytotoxic effects on normal and cancer cells, pointing towards different safety and efficacy profiles.

Testing of their LGR5 specific ADCs, anti-LGR5-vc-MMAE and anti-LGR5-NMS818, in two LGR5 expressing xenograft models with subcutaneous tumor formation, D5124 (human pancreatic cancer) and LoVoX1.1 (human colorectal cancer), revealed similar potency *in vivo*, resulting in tumor stasis or regression in both models. However, it remains unclear why the authors chose breast cancer (*in vitro* tests) and pancreatic cancer cell lines (xenograft model) instead of colorectal cancer cell lines as there are several publications, including our own work demonstrating an (over)expression of LGR5 in a plethora of widely used and commercially available colorectal cancer cell lines (21-23). LGR5 negative or low expressing colorectal cancer cell lines such as HCT 116 and LGR5 knockdown cell lines could have served as a negative control to ensure specificity of the anti-LGR5 ADCs.

Target-dependent safety assessment of their LGR5 specific ADCs revealed that rats treated with anti-LGR5-NMS818 showed severe gut and liver toxicities. Gut toxicity was characterized by blunted and/or fused intestinal villi including multifocal areas of crypt epithelial necrosis and decreased number of villi. Liver toxicity was characterized by acute, multifocal to coalescing, periportal to midzonal hepatocellular necrosis. Severe gut and liver toxicities were absent in control conjugate anti-HER2-NMS818 treated animals and in animals treated with MMAE conjugates, though both NMS818-conjugated ADCs (LGR5 and HER2) generated mild toxicological findings (bone marrow/hematologic and mild liver), typical of this class of drugs. Therefore, the severe liver and gut toxicities are dependent on targeting the NMS818-conjugated ADC to Lgr5 positive cells, indicating that when targeting Lgr5 positive cells the conjugated linker drug is critical for tolerability of the ADC. One possible explanation for the lack of gut toxicity with anti-LGR5-vc-MMAE is that the elimination of intestinal LGR5 positive cells is well tolerated under physiological conditions (17). In contrast, the anti-LGR5-NMS818 target-dependent toxicity observed in the intestine is more likely attributable to the combined elimination of LGR5 positive cells and their neighboring cells. Although both ADCs contain linkers that release a free membrane-permeable drug upon internalization,

according to the *in vitro* analyses the free drug released from anti-LGR5-NMS818 is much more potent on normal cells than that from anti-LGR5-vc-MMAE, thus probably affecting a larger number of cells. This increased damage of adjacent tissue could affect the dependence of the intestine on Lgr5 positive cells as it has been shown that Lgr5 positive intestinal stem cells are indispensable for intestinal regeneration after acute radiation-induced damage (18). The different liver toxicities could be explained by the fact that liver tissue is generally quiescent and therefore potentially more affected by anti-LGR5-NMS818, whose mechanism of action is independent from proliferation.

Finally, the authors assessed their better tolerated anti-LGR5 ADC anti-LGR5-vc-MMAE in a genetically engineered mouse model of intestinal tumorigenesis, the *Apc*$^{min/+}$;*Kras*$^{LSL-G12D/+}$;*Villin-Cre* (*AKV*) model, being relatively aggressive and harboring enhanced tumor multiplicity within the colon. Treatment of AKV mice with anti-LGR5-vc-MMAE resulted in a significantly extended survival along with a reduced tumor proliferation rate and a decrease of tumor size. However, the number of tumors was not changed compared to the control group. Of note, *Lgr5* mRNA expression was significantly elevated in intestinal tumors relative to matched normal mucosa, with a similar magnitude as previously reported in an inflammation driven mouse model of colonic tumorigenesis (AOM/DSS model) (21,24). Also, the fraction of Lgr5 positive tumor cells was comparable in the AKV model, in the AOM/DSS model (21,24) and in the *Lgr5*$^{EGFP-IRES-CreERT2}$/*Apc*$^{fl/fl}$/*R26R-Confetti* mouse model (12) of intestinal tumorigenesis and the fraction of Lgr5 positive tumor cells did not significantly differ from the fraction of Lgr5 positive normal intestinal stem cells per crypt. This suggests that Lgr5 positive tumor cells express a higher target antigen density at the cell surface than their normal counterparts do. Assuming that the process of ADC-antigen internalization is equally efficient in Lgr5 positive normal and tumor cells, intracellular concentration of the drug will be lower in normal cells due to a reduced amount of target antigens and the threshold concentration of the cytotoxic drug necessary to kill the cell might not be reached in normal cells, thus limiting toxicity to normal cells and enabling to target Lgr5 positive stem-like tumor cells more selectively.

Taken together, Junttila and colleagues could show that targeting of LGR5 positive colorectal cancer cells with ADCs is feasible without causing significant side effects to normal tissue in mice and rats, though linker and drug selection was critical to achieve acceptable toxicity profiles. Moreover, anti-LGR5 specific ADCs significantly prolonged survival in a genetically engineered mouse model of intestinal tumorigenesis, decreasing tumor size and reducing tumor growth rate. The number of tumors, however, remained unchanged. In LGR5 expressing xenograft models of colorectal cancer (LoVoX1.1) and pancreatic cancer (D5124), tumor growth was also reduced. Consistently, a study by Gong and colleagues published recently in *Molecular Cancer Therapeutics* reports that an LGR5 targeted ADC can eradicate gastrointestinal tumors and can prevent recurrence (25). Briefly, they generated two anti-LGR5 specific ADCs conjugating an anti-LGR5 specific antibody to MMAE via either a protease cleavable or non-cleavable chemical linker. *In vitro*, both anti-LGR5 ADCs effectively induced cytotoxicity in LGR5 high gastrointestinal cancer cell lines (AGS human gastric cancer cells and LoVo human colorectal cancer cells), but not in LGR5 negative (HCT15 human colorectal cancer cells) or LGR5 knockdown (LoVo shLGR5 human colorectal cancer cells) cancer cell lines, though the cleavable ADC exhibited higher potency *in vitro*. *In vivo*, the cleavable ADC was able to eradicate tumors and prevent recurrence in a xenograft model of colorectal cancer.

Altogether, targeting LGR5 positive cancer stem-like cells with ADCs represents an interesting potential therapeutic approach that should be further tested. Most of the data described here were obtained from cell lines or animal models in which Lgr5 expression and function has been extensively studied. By contrast, knowledge on the dependency of human intestinal homeostasis and colorectal cancer on LGR5 positive cells is limited. Thus, the fidelity of these findings remains to be established before clinical use.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Junttila MR, Mao W, Wang X, et al. Targeting LGR5+ cells with an antibody-drug conjugate for the treatment of colon cancer. Sci Transl Med 2015;7:314ra186.

2. Peters C, Brown S. Antibody-drug conjugates as novel anti-cancer chemotherapeutics. Biosci Rep 2015;35. pii: e00225.
3. Leushacke M, Barker N. Lgr5 and Lgr6 as markers to study adult stem cell roles in self-renewal and cancer. Oncogene 2012;31:3009-22.
4. Carmon KS, Gong X, Lin Q, et al. R-spondins function as ligands of the orphan receptors LGR4 and LGR5 to regulate Wnt/beta-catenin signaling. Proc Natl Acad Sci U S A 2011;108:11452-7.
5. de Lau W, Barker N, Low TY, et al. Lgr5 homologues associate with Wnt receptors and mediate R-spondin signalling. Nature 2011;476:293-7.
6. Barker N, Huch M, Kujala P, et al. Lgr5(+ve) stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro. Cell Stem Cell 2010;6:25-36.
7. Barker N, van Es JH, Kuipers J, et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 2007;449:1003-7.
8. Jaks V, Barker N, Kasper M, et al. Lgr5 marks cycling, yet long-lived, hair follicle stem cells. Nat Genet 2008;40:1291-9.
9. Ng A, Tan S, Singh G, et al. Lgr5 marks stem/progenitor cells in ovary and tubal epithelia. Nat Cell Biol 2014;16:745-57.
10. Barker N, Rookmaaker MB, Kujala P, et al. Lgr5(+ve) stem/progenitor cells contribute to nephron formation during kidney development. Cell Rep 2012;2:540-52.
11. Barker N, Ridgway RA, van Es JH, et al. Crypt stem cells as the cells-of-origin of intestinal cancer. Nature 2009;457:608-11.
12. Schepers AG, Snippert HJ, Stange DE, et al. Lineage tracing reveals Lgr5+ stem cell activity in mouse intestinal adenomas. Science 2012;337:730-5.
13. Sangiorgi E, Capecchi MR. Bmi1 is expressed in vivo in intestinal stem cells. Nat Genet 2008;40:915-20.
14. Takeda N, Jain R, LeBoeuf MR, et al. Interconversion between intestinal stem cell populations in distinct niches. Science 2011;334:1420-4.
15. Powell AE, Wang Y, Li Y, et al. The pan-ErbB negative regulator Lrig1 is an intestinal stem cell marker that functions as a tumor suppressor. Cell 2012;149:146-58.
16. Montgomery RK, Carlone DL, Richmond CA, et al. Mouse telomerase reverse transcriptase (mTert) expression marks slowly cycling intestinal stem cells. Proc Natl Acad Sci U S A 2011;108:179-84.
17. Tian H, Biehs B, Warming S, et al. A reserve stem cell population in small intestine renders Lgr5-positive cells dispensable. Nature 2011;478:255-9.
18. Metcalfe C, Kljavin NM, Ybarra R, et al. Lgr5+ stem cells are indispensable for radiation-induced intestinal regeneration. Cell Stem Cell 2014;14:149-59.
19. Huch M, Dorrell C, Boj SF, et al. In vitro expansion of single Lgr5+ liver stem cells induced by Wnt-driven regeneration. Nature 2013;494:247-50.
20. Ziskin JL, Dunlap D, Yaylaoglu M, et al. In situ validation of an intestinal stem cell signature in colorectal cancer. Gut 2013;62:1012-23.
21. Hirsch D, Barker N, McNeil N, et al. LGR5 positivity defines stem-like cells in colorectal cancer. Carcinogenesis 2014;35:849-58.
22. Uchida H, Yamazaki K, Fukuma M, et al. Overexpression of leucine-rich repeat-containing G protein-coupled receptor 5 in colorectal cancer. Cancer Sci 2010;101:1731-7.
23. McClanahan T, Koseoglu S, Smith K, et al. Identification of overexpression of orphan G protein-coupled receptor GPR49 in human colon and ovarian primary tumors. Cancer Biol Ther 2006;5:419-26.
24. Hirsch D, Hu Y, Ried T, et al. Transcriptome profiling of LGR5 positive colorectal cancer cells. Genom Data 2014;2:212-5.
25. Gong X, Azhdarinia A, Ghosh SC, et al. LGR5-Targeted Antibody-Drug Conjugate Eradicates Gastrointestinal Tumors and Prevents Recurrence. Mol Cancer Ther 2016;15:1580-90.

Cite this article as: Hirsch D, Ried T. Targeting colorectal cancer (stem-like) cells using LGR5 directed antibody drug conjugates. Ann Transl Med 2016;4(24):508. doi: 10.21037/atm.2016.11.78

