ONCOIMMUNOLOGY
2017, VOL. 6, NO. 10, e1345403 (3 pages)
https://doi.org/10.1080/2162402X.2017.1345403

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POINT OF VIEW

Labeling of cell therapies: How can we get it right?

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ABSTARCT

Labeling cells for non-invasive tracking *in vivo* using magnetic resonance imaging (MRI) is an emerging hot topic garnering ever increasing attention, yet it is fraught with numerous methodological challenges, which merit careful attention. Several of the current procedures used to label cells for tracking by MRI take advantage of the intrinsic phagocytic nature of cells to engulf nanoparticles, though cells with low intrinsic phagocytic capacity are also commonly studied. Before we take the next steps towards administering such cells *in vivo*, it is essential to understand how the nanolabel is recognized, internalized, trafficked and distributed within the specific host cell. This is even more critical when contemplating labeling of cells that may ultimately be applied *in vivo* to patients in a therapeutic context.

ARTICLE HISTORY
Received 3 May 2017
Revised 16 June 2017
Accepted 16 June 2017

KEYWORDS
Cell labeling; cell tracking; clinical application; electron microscopy; excess nanoparticles; internalization; intracellular; membrane-bound; MRI; NK cells; phagocytes; nanolabel; nanoparticles; patient studies; T cells

With reference to the article by Bouchlaka and colleagues in February last year,[1] this letter aims to reach out to the cell labeling and *in vivo* cell-tracking community, to stimulate an open discussion regarding the validity of cell labeling protocols. Particular consideration is given to the labeling of cells that will ultimately find their way to clinical application.

Labeling cells for non-invasive tracking *in vivo* using magnetic resonance imaging (MRI) is an emerging hot topic garnering ever increasing attention, yet it is fraught with numerous methodological challenges which merit careful attention. Several of the current procedures used to label cells for tracking by MRI take advantage of the intrinsic phagocytic nature of cells to engulf nanoparticles (also referred to as nanoemulsions, nanodroplets, nanolabels, particles). The labeling of cell therapies with nanoparticles detectable with imaging methods such as MRI has been particularly vital for tracking them following *in vivo* application, in both animal[2] and patient[3,4] studies. Other studies, including Bouchlaka et al.[1] extend this general approach to label cells with low intrinsic phagocytic capacity. Before we take the next steps toward administering such cells *in vivo*, it is essential to understand how the nanolabel is recognized, internalized, trafficked and distributed within the specific host cell.[5] This is even more critical when contemplating labeling of cells that may ultimately be applied *in vivo* to patients in a therapeutic context. Therefore we need to ask the right questions regarding the specific cell type to be labeled, and the methods used before transfer of the cells into living organisms. Most research groups are aware of the possible impact of nanoparticle labeling on cellular function. This is a very valid point and has also been considered in the present study.[1] However, 2 other equally important and simple issues that are highly relevant to cell tracking are unfortunately often neglected.

The first issue concerns the method used to effectively remove unbound surplus nanoparticles from the cell cultures, before their adoptive transfer *in vivo*. It is common laboratory practice to use a series of centrifugation and washing steps to remove cell debris or undesirable soluble factors from cell culture media. This approach is also often used to remove excess nanolabel from the culture and has also been used in this study.[1] Here special caution should be taken with regard to the type of labeling nanoparticle used as well as the type of cell being studied. For example, high-density nanoemulsions sediment equally as well as some cells — particularly small immune cells such as T cells and NK cells — following simple centrifugation steps. Thus simple centrifugation and washing may not be reliably effective to remove excess nanoparticles from the cell culture, unless low-sedimentation rate nanoemulsions are prepared.[6] Furthermore, differences between suspension and adherent cells cannot be neglected — excessive nanoparticles might prove easier to remove from the latter; due to the adhesion of the cells to the culture plates (although a level of adhesion of the nanoparticles to the culture plates should not be disregarded). When dealing with particularly dense nanoparticles, the use of density gradient centrifugation or cell sorting may be the best solution for clearing labeled cells from unbound nanoparticles, since particles and cells can then be sorted according to size, density and morphology or — in the case of cell sorting — cell-specific markers. Nevertheless, the number of cells lost using these

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Commentary to: Myriam N. Bouchlaka, Kai D. Ludwig, Jeremy W. Gordon, Matthew P. Kutz, Bryan P. Bednarz, Sean B. Fain & Christian M. Capitini (2016): 19F-MRI for monitoring human NK cells *in vivo*, OncoImmunology, DOI: 10.1080/2162402X.2016.1143996

approaches could be an important consideration, especially when dealing with small populations of rare cell types, where cell numbers may be limited.

The second issue concerns the cell imaging technique used to confirm that the cells have indeed been labeled. Electron microscopy is the gold standard for definitive confirmation of intracellular localization of nanoparticle labels, and is the method of choice.[7-9] With its high resolution, electron microscopy can clearly visualize the intracellular compartments with high definition, and visualization of the nanoparticles with this method provides the best assurance of successful cell labeling. Confocal microscopy is commonly used to assess uptake of fluorescently-labeled nanoparticles, and while this is a useful tool most researchers often omit the necessary additional staining steps to detail the plasma membrane, intracellular compartments and cytoskeleton. These added steps are vital to accurately discriminate between true intracellular labeling and membrane-bound labeling. In the present study fluorine (^{19}F) MR spectroscopy (MRS) was performed to determine labeling efficiency of ^{19}F-labeled NK cells,[1] which is fine, especially if the first issue considering removal of unbound surplus nanoparticles is completely under control. If unbound nanoparticles are still present in the cell suspension, these will however also be present in the cell lysate used during ^{19}F MRS analyses.

There are also several other issues that need to be considered. With reference to actively dividing cells such as T cells, one question to keep in mind is whether the label will be passed on to the progeny. Will the MR signal in daughter cells be half that of the mother cells, as would be expected when labeling with intracellular fluorescent dyes for flow cytometric measurements? Furthermore, for cells and their progeny that are likely to persist *in vivo*, cell viability and function tests should be performed several days, even weeks (if possible) after nanoparticle labeling and clearing. To study the migration of proliferating cells *in vivo*, the implementation of reporter genes for optical imaging,[10] nuclear medicine[11] or MRI[12] are set to play an important role in molecular imaging.[13]

While reference is made to the study by Bouchlaka et al. published in this journal, several other studies have reported the use of the same principle to label cells with an innately low phagocytic and high proliferative potential.[1,14] This is by no means meant as a criticism to any particular study that has labeled cells other than those that are naturally phagocytic to track them by MRI. Rather, we merely wish to sound a cautionary note to all research groups, including our own, that particular attention should be given to cell labeling procedures in general, especially before application of the labeled cells into living organisms. While confirmation of intracellular localization of nanoparticles by electron microscopy may not be required for every individual experiment, it should at the least be done for the first reports of labeling new cell types with new nanoparticle formulations. The worst case scenario is that unlabeled cells are transferred together with contaminating unbound nanoparticles that have not been thoroughly removed from the cell suspension into living organisms — the ensuing *in vivo* MRI images might be completely misrepresentative. While some may regard the basic research behind cell labeling as an upstream process that needs to be tackled just once, the reality is that labeling strategies are continuously being developed and optimized, and different cell types will behave differently with the particular nanoparticle mélange available.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Acknowledgement

This work has been supported in part by positive discussion through A FACTT network (Cost Action BM1305: www.afactt.eu). COST is supported by the EU Framework Programme Horizon 2020.

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