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Intraoperative Near-infrared Fluorescent Cholangiography (NIRFC) in Mouse Models of Bile Duct Injury: Reply

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We are grateful to Ishizawa et al. for sharing their clinical perspective and experience using indocyanine green (ICG) for biliary imaging. The authors raise some interesting points, which we address below.

Near-infrared (NIR) imaging has become an important minimally invasive, surgical and preclinical tool. This is so because light in the 650–900 nm window traverses tissue more effectively than light in the visible range, and also because less autofluorescence occurs in this region [1]. Ultimately, the goal for NIR imaging is to use multiple fluorescent probes in the same way as multicolor imaging (fluorescent intravital live microscopy, FILM), to report on a variety of anatomic, physiologic, or molecular events during real-time surgical intervention [2]. The maximum tissue depth that can be visualized in the NIR by epifluorescence is a complex function that depends on many factors, including target-to-background ratios, charge-couple device (CCD) quantum efficiencies and dark noise, absolute concentrations of the fluorophore, type of fluorochrome, properties of the imaging system (including magnification, numerical aperture, fluorescence, and excitation filters), the excitation wavelength, and tissue optical properties, among other factors. Most systems attempt to work in the 680/700 nm (excitation/emission) to 800/850 nm range; and in the past, in vivo imaging at both ends of this range has been successful, even for deep tomographic imaging [2]. The video frame rate of NIR cameras has to be adjusted so that it collects enough photons to capture a high-quality image in the shortest period of time. The faster the frame rate, the better it is at freezing motion, but this effect results in images that have lower signal-to-noise ratios and therefore reduced image quality. This limitation can be easily overcome, however, by increasing the power of the excitation light (e.g., using a laser beam or a light-emitting diode) or by choosing a different camera model. Indeed, the CCD field is advancing rapidly, and newer models with these attributes are continuing to emerge.

Indocyanine green (ICG) is clinically approved and has unique applications for imaging, based on its pharmacokinetics. Indocyanine green is approved for clinical use and is a relatively safe dye [3]. It is a pentamethine fluorochrome without handles for conjugation and bears only two sulfonates, which makes it reasonably lipophilic and plasma protein avid. Compared to newer cyanine fluorochromes, it is generally less "bright" [4–7]. Its vascular half-life is approximately 3 min, and it is excreted predominantly via the liver. Given its broad tissue distribution, it is used primarily for determining cardiac output, hepatic function and perfusion, or in ophthalmic imaging. However, ICG is also beginning to be employed for other applications, such as in visualizing atherosclerotic segments [8], coronary artery bypass surgery [9], colonoscopy, and biliary surgery [10].

Where and when does ICG not work well and what are the alternatives? Although ICG is being applied to biliary imaging, it does not have ideal pharmacokinetic properties for imaging acute biliary injury or intraoperative surgical complications, such as unintentional leaks, ductal injury, or accidental ligature. After intravenous injection, ICG often requires more than 20 min to appear in the biliary tract in sufficient quantity for measurement [11]. It also leads to significant hepatic fluorescence and therefore achieves only a limited target-to-background ratio. Ideal biliary NIR

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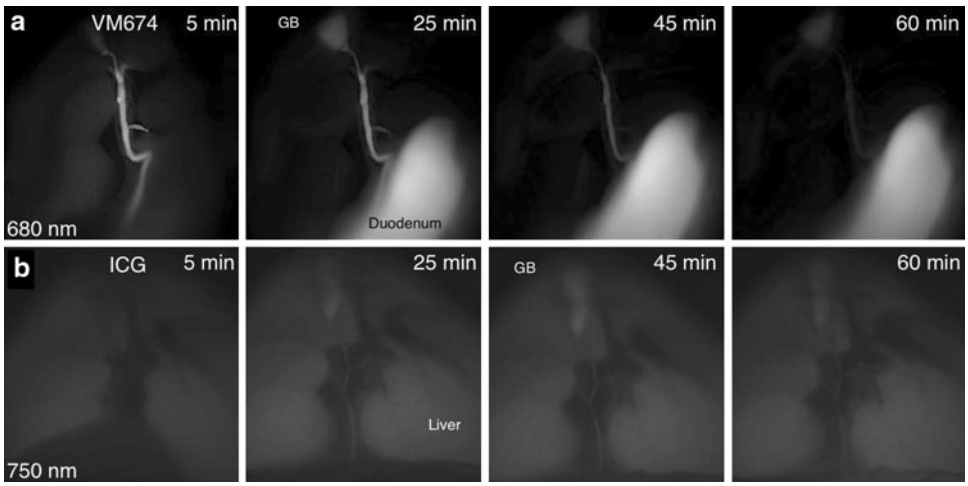

Fig. 1 Imaging the biliary duct: a comparison of the VM674 fluorochrome (**a**) with indocyanine green (ICG) (**b**). Note the much better delineation of the biliary anatomy immediately after intravenous injection of VM674. Identical windowing

agents need to have fast (minutes) and high biliary excretion for them to maximize target-to-background ratios in the biliary system after intravenous injection. The VM674 fluorochrome described in our article was designed and developed iteratively, so as to achieve these ideal properties. As shown in Fig. 1, compared to ICG, VM674 has a far better target-to-background ratio and yields crisper images of the structures of interest. In addition, VM674 enables easier identification of smaller leaks and/or can be used at lower doses. The goal of our study was to image bile duct injuries in real-time, i.e. at the time complications occur during surgery rather than in a chronic setting. Thus, the use of ICG would not be entirely appropriate in these scenarios unless it is given to every patient in advance of surgery.

In summary, we appreciate the authors' comments and hope that their clinical success and emerging new technologies/agents (in addition to those developed by our and similar teams) will spawn an era of sophisticated, minimally invasive tools that will be of great benefit to our patients.

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