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Predicting the influence of liposomal lipid composition on liposome size, zeta potential and liposome-induced dendritic cell maturation using a design of experiments approach

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1 Predicting the influence of liposomal lipid composition on liposome size, zeta potential and 2 liposome-induced dendritic cell maturation using a design of experiments approach 3 *Peter C. Soema ¹, Geert-Jan Willems ¹, Wim Jiskoot ², Jean-Pierre Amorij ¹, Gideon Kersten ^{1,2} 4 5 6 ¹ Intravacc (Institute for Translational Research), Bilthoven, The Netherlands 7 ² Division of Drug Delivery Technology, Leiden Academic Centre for Drug Research, Leiden University, 8 Leiden, The Netherlands 9 10 *Corresponding author: 11 Peter Soema 12 Antonie van Leeuwenhoeklaan 9 13 3721 MA Bilthoven, The Netherlands 14 peter.soema@intravacc.nl 15 16 **Abstract** 17 In this study, the effect of liposomal lipid composition on the physicochemical characteristics and 18 adjuvanticity of liposomes was investigated. Using a design of experiments (DoE) approach, peptide-19 containing liposomes containing various lipids (EPC, DOPE, DOTAP and DC-Chol) and peptide 20 concentrations were formulated. Liposome size and zeta potential were determined for each 21 formulation. Moreover, the adjuvanticity of the liposomes was assessed in an in vitro dendritic cell 22 (DC) model, by quantifying the expression of DC maturation markers CD40, CD80, CD83 and CD86. 23 The acquired data of these liposome characteristics were successfully fitted with regression models, 24 and response contour plots were generated for each response factor. These models were applied to

predict a lipid composition that resulted in a liposome with a target zeta potential. Subsequently, the

expression of the DC maturation factors for this lipid composition were predicted and tested in vitro;

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