Will detection of pre-existing (baseline) NS5A RAVs with high-fold *in vitro* resistance be another predictor in treatment decision?

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Treatment emergent NS5A RAVs in different genotypes

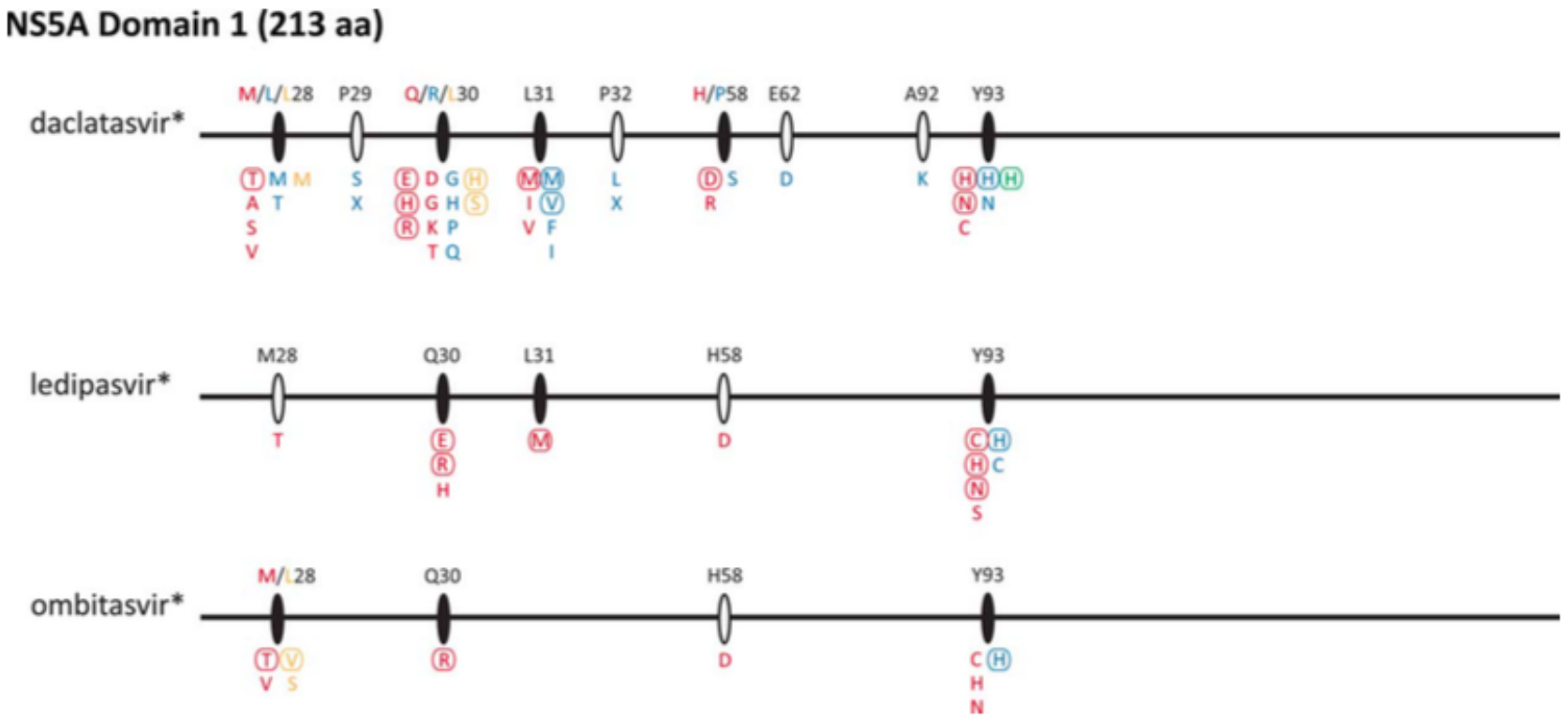

Fig. 3. NS5A resistance-associated substitutions observed with treatment. *Compounds approved for clinical use. Amino acid deletions are designated with an *X*. Substitutions are color-coded based on genotype and subtype: 1a, red; 1b, blue; 2, brown; 3a, green; 4, orange (daclatasvir 4, ombitasvir 4d). See Fig. 1 legend.

Potential clinical relevant NS5A RAVs in GT 1a, 1b, 3a

(Lontok et al. Hepatology, 2015; Sarrazin. Journal of Hepatology 2015)

- *in vitro* fold-resistance with different GTs
- prevalence at baseline with population sequencing (cut-off 20%)

RAV	Approx. Prevalence at baseline [m, n, o , p]	Fold-Change in Resistance Compared to Wild-Type Replicon*		
		Daclatasvir (DCV)	Ombitasvir (OMV)	Ledipasvir (LDV)
M28T	1.2 % (GT 1a)	683 (GT 1a)[a]	8965 (GT1a)[b]	61 (GT 1a)[c]
M28V	3.5 % (GT 1a)	1.3 (GT 1a)[i]	58 (GT 1a)[c]	NDA
Q30E		7500 (GT 1a)[a]	NDA	5458 (GT 1a) [c]
Q30H	0.6 % (GT 1a)	1450 (GT 1a)[a]	3 (GT 1a)[j]	183 (GT 1a)[c]
Q30R		1217 (GT 1a)[a] and 1 (GT 1b)[a]	800 (GT1a)[b]	632 (GT 1a) [c]
A30K	5 % (GT 3a)	20-100 (GT 3a)	NDA	NDA
L31M	1.4 % GT 1a), 4 % (GT 1b)	350 (GT 1a)[a] and 3 (GT 1b)[i]	2 (GT 1a)[k] and 0.9 (GT 1b)[k]	554 (GT 1a) [c] and 2,5-10 (GT 1b) [d]
L31V		3350 (GT 1a) and 23 (GT 1b)[a]	8 (GT 1b)[f]	100-1000 (GT 1a) [d]
H58D	< 1% (GT 1a)	483 (GT 1a)[e]	243 (GT1a)[b]	>1127 (GT 1a) [c]
Y93C	0.6 % (GT 1a)	1850 (GT 1a) and 3.5 (GT 1b)[e]	1675 (GT1a)[b]	>1602 (GT 1a) [c]
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Y93N	1 % (GT 1a) 0.7 % (GT 1b)	47017 (GT 1a) and 28 (GT1b)[a]	66740 (GT1a)[b]	>14706 (GT 1a) [c]

*HCV replicon phenotype results between different drugs should be interpreted with caution as data were generated by different assay conditions

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Genotype 1a

Potential relevant baseline NS5A RAVs with > 100 fold in resistance**

RAV	Prevalence at baseline (cut off 20 %)	In vitro Fold-Change in Resistance*			Notes
		DCV	OMV	LDV	Reduced SVR with RAVs at baseline
M28T	1.2 %	683	8965	61	
L31M	1.4 %	350	2	554	80 % SVR, Lawitz EASL 2015 Harvoni retreatment 24 w
Y93C	0.6 %	1850	1675	>1602	
Y93H	1 %	5367	41383	3309	33% SVR Lawitz EASL 2015 Harvoni retreatment 24 w.
Y93N	1 %	47017	66740	>14706	33 % SVR Lawitz EASL 2015 Harvoni retreatment 24 w. Also observed in AVIATOR study for two relapsers.

*HCV replicon phenotype results between different drugs should be interpreted with caution as data were generated by different assay condition.

**These RAVs seem also to effect Elbasvir.

Genotype 1b

Potential relevant baseline NS5A RAVs with > 100 fold in resistance

RAV	Prevalence at baseline (cut off 20 %)	In vitro Fold-Change in Resistance*			Notes
		DCV	OMV	LDV	
Y93H	9 %	19	77	1319	

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Broad cross-resistance with "early generation" NS5As

Wyles D, AASLD 2015

Fold-change	1a				1b	
	M28T	Q30R	L31M/V	Y93H/N	L31V	Y93H/N
LDV	20x	>100x	>100x/ >100x	>1,000x/ >10,000		>100x/--
Ombitasvir	>1000x	>100x	<3x / >100x	>10,000x/ >10,000x	<10x	20x/50x
DCV	>100x	>1000x	>100x/ >1000x	>1,000x/ >10,000x	<10x	20x/50x
Elbasvir	20x	>100x	>10x / >100x	>1,000x/ >1,000x	<10x	>100x/--
Velpatasvir	<10x	<3x	20x/50x	>100x/ >1000x		<3x/--
ACH-3102	30x	20x	<10x	>100x/>100x		<3x/<3x
ABT-530	<3x	<3x	<3x	<10x/<10x	<3x	<3x/<3x
MK-8408	<10x	<10x	<10x	<10x	<10x	<10x

Wang C. AAC 2012. Cheng G. #1172. EASL 2012. Zhao Y. #A845 EASL 2012. Yang G. EASL 2013. Ng T. #639 CROI 2014. Asante-Appiah E. AASLD 2014.

Genotype 3a

Potential relevant baseline NS5A RAVs with > 100 fold in resistance

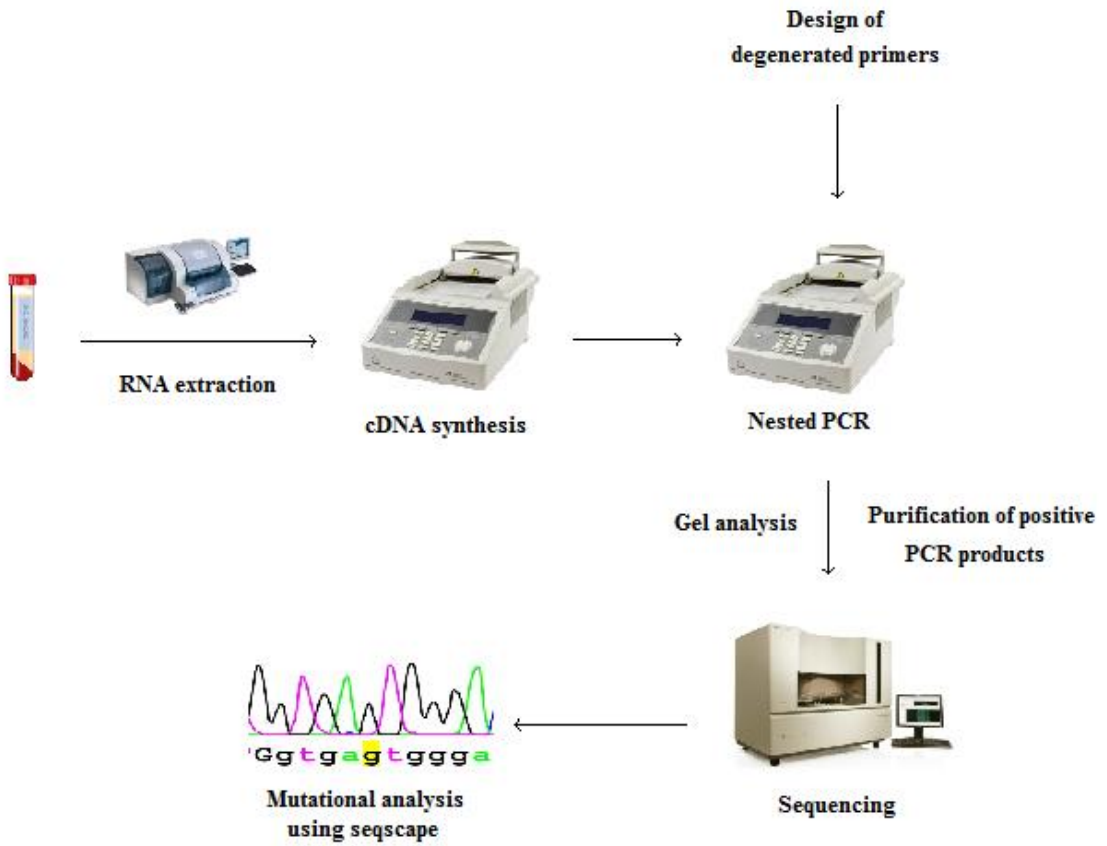
RAV	Prevalence at baseline (cut off 20 %)	In vitro Fold-Change in Resistance			Notes
		DCV	OMV	LDV	Reduced SVR with RAVs at baseline
A30K	5 %	20 - 100	NDA	NDA	Lower SVR only observed with DCV + PEG-INF/RBV ALLY 3+ study AASLD 2015 12 /16 w + RBV; 7 of 49 had A30K at baseline ; no relapse
Y93H	8 %	2154	NDA	NDA	33% SVR with DCV+SOF 12 w for patients with cirrhosis, and 67 % SVR for patients without cirrhosis. ALLY 3 study; Nelson e al Hepatology 2015 ; 13 0f 147 had Y93H at baseline. ALLY 3+ study AASLD 2015, 12 /16 w + RBV; only 2 of 49 had Y93H at baseline; one relapsed.

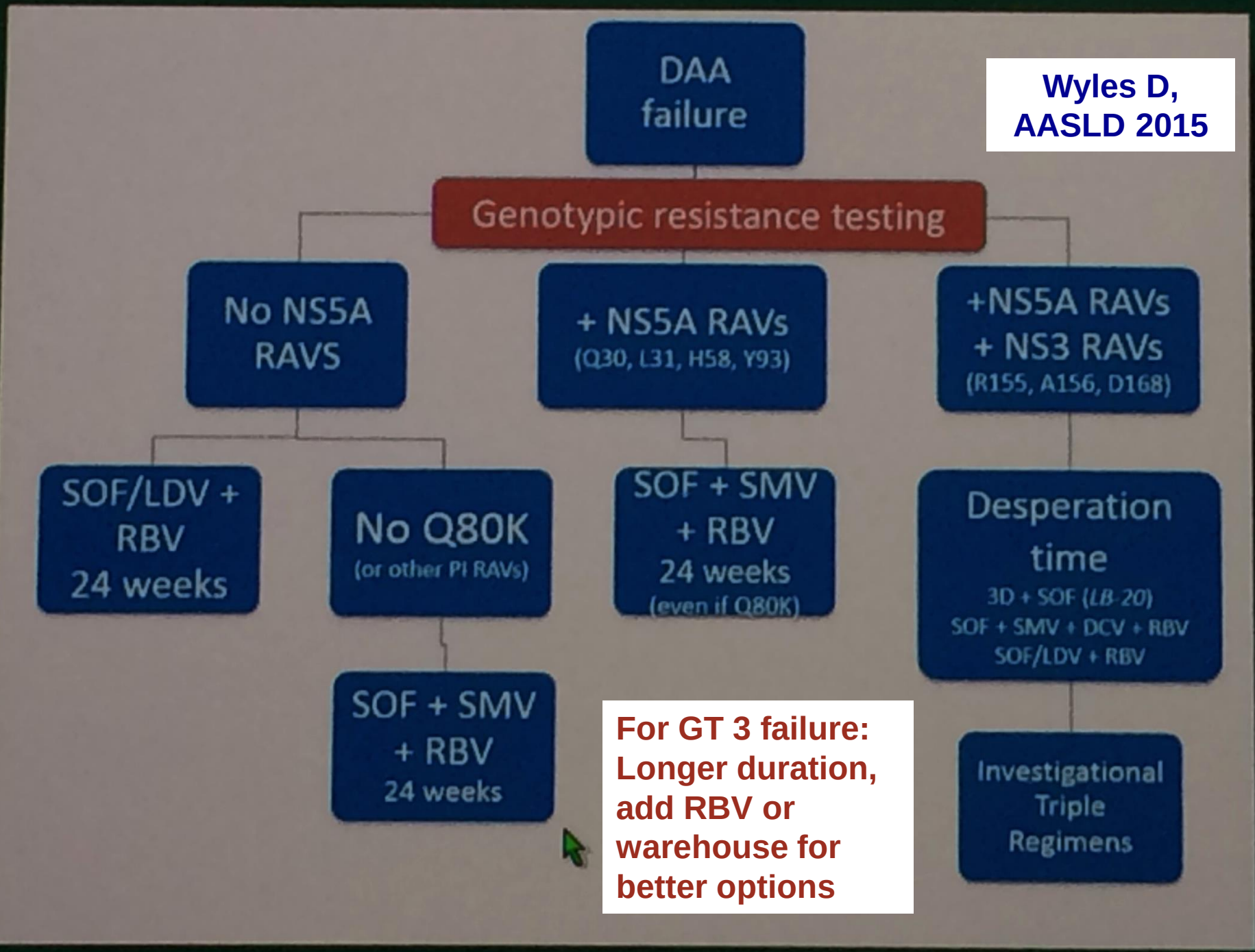
NS5A population based sequencing method at Clinical Microbiology Uppsala

Routine method since Dec 2014 *(200 € per sample; Method published: Lindstrom et al Infectious Diseases 2015)*

Second line treatment recommendation for NS5A RAVs (>100 fold) at treatment failure

Plus <u>modest</u> first line treatment recommendations for NS5A RAVs (>100 fold) at baseline




DAA failure
Wyles D, AASLD 2015
Genotypic resistance testing
No NS5A RAVS
+ NS5A RAVs (Q30, L31, H58, Y93)
+NS5A RAVs + NS3 RAVs (R155, A156, D168)
SOF/LDV + RBV 24 weeks
No Q80K (or other PI RAVs)
SOF + SMV + RBV 24 weeks (even if Q80K)
Desperation time
3D + SOF (LB-20)
SOF + SMV + DCV + RBV
SOF/LDV + RBV
SOF + SMV + RBV 24 weeks
For GT 3 failure: Longer duration, add RBV or warehouse for better options
Investigational Triple Regimens

Nordic multicenter study 2015 – 2016

Aim:

To explore if baseline NS5A RAVs with high fold resistance may predict treatment outcome, together with other negative factors (such as high fibrosis stage, Treatment Exp, high viral load, IL28B non-CC etc)

To find more cost-efficient combination of DAA treatment, and reduce the treatment duration for an HCV infected individual

In collaboration with:

Hege Kileng and Tore Gutteberg, Tromsö, Norway

Anders Lannergård, Uppsala, Sweden

Lars Wesslèn, Gävle, Sweden

Ann-Sofi Duberg, Örebro, Sweden

Astrid Danielsson, Falun, Sweden

Soo Aleman, Stockholm

Magnhild Gangsoy, Bodö, Norway

Intervention arm:
Uppsala, Tromsö and Gävle
Preform and adjust to baseline RAVs before treatment choice

Control arm:
Örebro, Falun, Bodö and Stockholm
Treats conventional i.e. without baseline before treatment choice

Aim to have in each arm (including SVR results) by end of 2016:
100 GT 1a, 40 GT 1b and 100 GT 3a patients
Which also reflect the normal distribution in Sweden and Norway

Preliminary results Oct 2015 in Intervention arm of prevalence at baseline:

RAV	**Uppsala**	**Tromsö**
M28V	14% (GT 1a, n=43)	15% (GT 1a, n=27)
A30K	2% (GT 3a, n=38)	4% (GT 3a, n=20)
Y93H	0% (GT 1a, n=43)	4% (GT 1a, n=27)
Y93H	10% (GT 1b, n=10)	0% (GT 1b, n=5)
Y93H	8% (GT 3a, n=38)	10% (GT 3a, n=20)

SVR results with NS5A combination treatment:
1 relapse in Uppsala and 2 in Tromsö, all GT 3a, F4 and no baseline RAVs
No results yet from Control arm

Thank you!

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a (Fridell et al., 2010), b (Williams et al., 2012), c (Lontok et al. 2015), d (Dvory-Sobol et al. 2014),e (Wang et al., 2012), f (DeGoey et al., 2014), g (Paolucci et al., 2013), h (Hernandez et al., 2013), i (Fridell et al., 2011) j (Krishnan et al., 2015), k (Krishnan et al., 2014), l (Lawitz et al., 2012), m (Dietz et al 2015) n (Nelson et al 2015) o (Sarrazin 2015) p (Lindström et al 2015)