Burnet Institute
Medical Research. Practical Action.

Hepatitis C virus can accumulate mutations that confer resistance to a novel broadly neutralizing antibody MAb24

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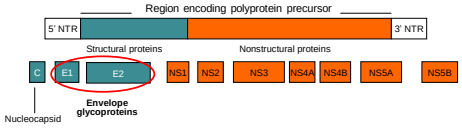
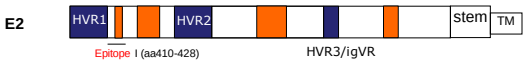
HCV

- Genetic diversity: difficult to treat and prevent
  - 7 genotypes; >50 subtypes
  - High mutation rate→quasispecies
- Treatment
  - Direct acting antivirals (DAAs)
- Immunotherapeutics for HCV treatment and prevention
  - MAbs: well tolerated; ideal for HCV patients with liver transplants



E2 and E2-specific antibodies

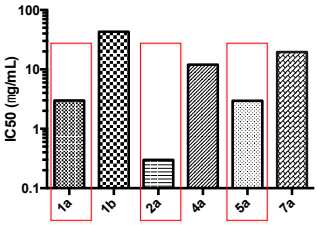
- Heterodimerize with glycoprotein E1 via TMD
- Direct interaction with host cell receptor CD81
- Key antigenic component in antibody response
  - Neutralizing antibodies to E2 are protective *in vitro* and *in vivo*
  - HVR1 elicits isolate specific NAb
  - Epitope I elicits brNAbs
- Delta3: E2 with three HVRs deleted; lead candidate for a universal HCV vaccine.



MAb24 (HCVD123.6E11.1B4.1E3)

- Mouse monoclonal antibody
- Elicited to Delta3 immunization
- Cross-reacts with $E2_{661}$ proteins from 6 major genotypes
- Inhibit E2-CD81 interaction (IC_{50} 3μg/mL)

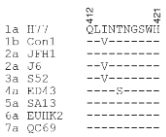
MAb24 is a broadly neutralizing antibody and can cross-neutralize HCV from different genotypes



- Representative of broadly NAbs elicited by Delta3
- Potential as immunotherapeutic

MAb24 epitope mapping

- Epitope I (residues 412-421)
  - Highly conserved across genotypes
  - Target of brNAbs (AP33, 3-11, HCV1)
  - Low prevalence (2.3%) in HCV infected people



	Q^{412}	L^{413}	I^{414}	N^{415}	T^{416}	N^{417}	G^{418}	S^{419}	W^{420}	H^{421}
MAb24 (mouse)										
AP33 (mouse)										
3-11 (rat)										
HCV1 (human)										

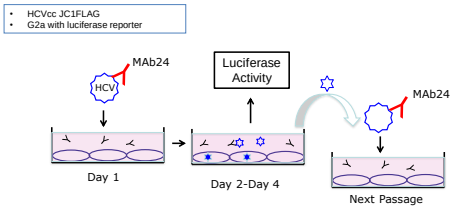
Aim

- To determine whether HCV can generate MAb24 resistance *in vitro*

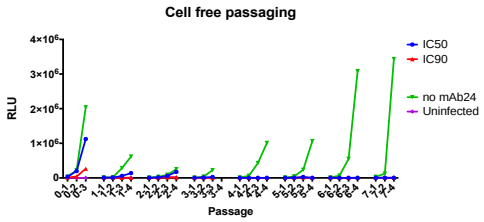
Methods

To generate MAb24^{R} virus, we passaged the virus with MAb24, either in **cell-free manner** or **with infected cells**

1. Cell-free Passaging of HCV in the presence of MAb24



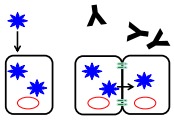
Virus was unable to generate escape mutants when passaged in the cell-free methods

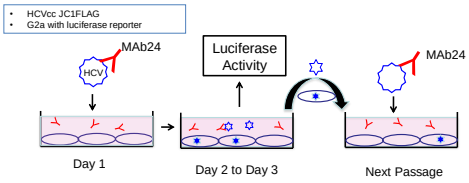


MAb24 potently inhibits cell-free transmission of HCV

2. Passaging with infected cells with MAb24

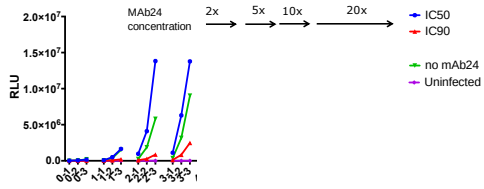
- HCV alternative transmission mode
  - Cell-cell spread
    (Timpe et al.,2008; Brimacombe et al., 2011)
- Advantages
  - Rapid dissemination
  - Immune evasion





Passaging with **infected cells**
- Serves as a source for cell-cell spread; In vivo relevance

Passaging with infected cells enabled the rapid emergence of MAb24 resistant virus



- P9 virus was able to replicate in the presence of MAb24 at **196µg/mL**
- P9 virus can confer MAb24 resistance and replicate in the absence of MAb24

Mutations associated with MAb24 resistance

- Sequence analyses on E1E2 structural region from cell-free P9 virus
  - No changes in E1
  - Mutations accumulated exclusively in MAb24 epitope region (epitope I)

- Mutations
  - N415D (71%)
  - N417S (29%)

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                    Ψ
2a J6 WT      Q412LVNTNGSWH421
MAb24R(71%)   ---D------
MAb24R(29%)   -----S-----
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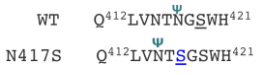
Mutations associated with MAb24 resistance

N415D

•Residue N415: important in MAb24 epitope recognition
•disrupt the β loop stability of epitope I (Kong et al., 2012)
•Enhance CD81 binding (Dhillon et al., 2010)

N417S

- glycan shift from N417 to N415 (Pantua et al., 2013)
- Shield MAb24 epitope by glycan modification



Mutations have been described in chimpanzees treated with brNAb HCV1 (Morin et al., 2012)

Summary

- In vitro evidence that HCV can acquire MAb24 resistance by directly modifying epitope residues
- Combination immunotherapies needed
  – MAbs that target different epitope regions of E2

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