Characterization of IDH1 p.R132H Mutant Clones Using Mutation-specific Antibody in Myeloid Neoplasms

汇 报 人：克祯彧
指导教授：徐玉乔

Key words

- AML： 急性髓系白血病
- AML-NOS： AML 非特指型
- AML-MRC： AML 伴骨髓增生异常相关改变
- t-AML： 治疗相关髓系肿瘤
- MDS： 骨髓增生异常综合征
- MPN： 骨髓增殖性肿瘤
- MDS/MPN： 骨髓增生异常/骨髓增殖性肿瘤
- MPN-BP： 骨髓增殖性肿瘤急变期
- MAF： 最小突变等位基因频率
- MRD： AML 伴微小残留

MAF (Minor Allele Frequency)

- 指一个突变位点的最小突变频率
- MAF 广法应用于复杂疾病的全基因组关联研究
- MAF 越大越好，较小的 MAF 将会使统计效能降低，从而造成假阴性的结果
- 通常认为一个位点的 MAF>0.05 才有研究的意义
- MAF 还可以估算样本量或检验效能，并可以确定基因型的频率

造血与淋巴组织肿瘤 WHO 分类一览表

骨髓增殖性肿瘤
慢性粒细胞白血病，*BCR – ABL1* 阳性 9875/3
慢性中性粒细胞白血病 9963/3
真性红细胞增多症 9950/3
原发性骨髓纤维化 9961/3
原发性血小板增多症 9962/3
慢性嗜酸粒细胞白血病，非特指型 9964/3
肥大细胞增生症
皮肤肥大细胞增生症 9740/1
系统性肥大细胞增生症 9741/3
肥大细胞白血病 9742/3
肥大细胞肉瘤 9740/3
皮肤外肥大细胞瘤 9740/1
骨髓增殖性肿瘤，不能分类 9975/3

髓系和淋系肿瘤伴嗜酸粒细胞增多和 PDGFRA，PDGFRB 或 FGFR1 异常
髓系和淋系肿瘤伴 *PDGFRA* 重排 9965/3
髓系肿瘤伴 *PDGFRB* 重排 9966/3
髓系与淋系肿瘤伴 *FGFR1* 异常 9967/3

骨髓增生异常/骨髓增殖性肿瘤
慢性粒 – 单核细胞白血病 9945/3
不典型慢性髓系白血病，*BCR – ABL1* 阴性 9876/3
幼年型粒 – 单核细胞白血病 9946/3
骨髓增生异常/骨髓增殖性肿瘤，不能分类 9975/3
难治性贫血伴环形铁粒幼细胞(RARS)
及血小板显著增多(RARS – T) 9982/3

骨髓增生异常综合征
难治性血细胞减少伴单系发育异常
难治性贫血 9980/3
难治性中性粒细胞减少 9991/3
难治性血小板减少 9992/3
难治性贫血伴环形铁粒幼细胞 9982/3
难治性血细胞减少伴多系发育异常 9985/3
难治性贫血伴原始细胞过多 9983/3
骨髓增生异常综合征伴孤立性 5q 9986/3
骨髓增生异常综合征，不能分类 9989/3
儿童骨髓增生异常综合征
儿童难治性血细胞减少 9985/3

急性髓系白血病(AML)和相关前体肿瘤
AML 伴重现性遗传学异常
AML 伴 t(8;21)(q22;q22)；
RUNX1 – RUNX1T1 9896/3
AML 伴 inv(16)(p13.1q22)或 t(16;16)
(p13.1;q22)；*CBFB – MYH11* 9871/3
APL 伴 t(15;17)(q22;q12)；
PML – RARA *9866/3*
AML 伴 t(9;11)(p22;q23)；
MLLT3 – MLL • *9897/3*
AML 伴 t(6;9)(p23;q34)；
DEK – NUP214 *9865/3*
AML 伴 inv(3)(q21q26.2)或 t(3;3)
(q21;q26.2)；RPN1 – EVI1 9869/3
AML(原巨核细胞性)伴 t(1;22)
(p13;q13)；*RBM15 – MKL1* 9911/3
急性髓系白血病伴 NPM1 突变 9861/3
急性髓系白血病伴 CEBPA 突变 9861/3

急性髓系白血病伴骨髓增生异常 – 相关改变 9895/3
治疗相关髓系肿瘤 9920/3
急性髓系白血病，非特指型 9861/3
AML，微分化型 9872/3
AML，无成熟迹象型 9873/3
AML，有成熟迹象型 9874/3
急性粒 – 单核细胞白血病 9867/3
急性原单核细胞和急性单核细胞白血病 9891/3
急性红白血病 9840/3
急性巨核细胞白血病 9910/3
急性嗜碱粒细胞白血病 9870/3
急性全髓增殖症伴骨髓纤维化 9931/3

Myeloproliferative neoplasms

Chronic myeloid leukaemia, *BCR-ABL1*–positive	9875/3
Chronic neutrophilic leukaemia	9963/3
Polycythaemia vera	9950/3
Primary myelofibrosis	9961/3
Essential thrombocythaemia	9962/3
Chronic eosinophilic leukaemia, NOS	9964/3
Myeloproliferative neoplasm, unclassifiable	9975/3

Mastocytosis

Cutaneous mastocytosis	9740/1
Indolent systemic mastocytosis	9741/1
Systemic mastocytosis with an associated haematological neoplasm	9741/3
Aggressive systemic mastocytosis	9741/3
Mast cell leukaemia	9742/3
Mast cell sarcoma	9740/3

Myeloid/lymphoid neoplasms with eosinophilia and gene rearrangement

Myeloid/lymphoid neoplasms with *PDGFRA* rearrangement	9965/3
Myeloid/lymphoid neoplasms with *PDGFRB* rearrangement	9966/3
Myeloid/lymphoid neoplasms with *FGFR1* rearrangement	9967/3
Myeloid/lymphoid neoplasms with PCM1-JAK2	9968/3*

Myelodysplastic/myeloproliferative neoplasms

Chronic myelomonocytic leukaemia	9945/3
Atypical chronic myeloid leukaemia, *BCR-ABL1*–negative	9876/3
Juvenile myelomonocytic leukaemia	9946/3
Myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis	9982/3
Myelodysplastic/myeloproliferative neoplasm, unclassifiable	9975/3

Myelodysplastic syndromes

Myelodysplastic syndrome with single lineage dysplasia	9980/3
Myelodysplastic syndrome with ring sideroblasts and single lineage dysplasia	9982/3
Myelodysplastic syndrome with ring sideroblasts and multilineage dysplasia	9993/3*
Myelodysplastic syndrome with multilineage dysplasia	9985/3
Myelodysplastic syndrome with excess blasts	9983/3
Myelodysplastic syndrome with isolated del(5q)	9986/3
Myelodysplastic syndrome, unclassifiable	9989/3
Refractory cytopenia of childhood	9985/3

Myeloid neoplasms with germline predisposition

Acute myeloid leukaemia with germline *CEBPA* mutation	
Myeloid neoplasms with germline *DDX41* mutation	
Myeloid neoplasms with germline *RUNX1* mutation	
Myeloid neoplasms with germline *ANKRD26* mutation	
Myeloid neoplasms with germline *ETV6* mutation	
Myeloid neoplasms with germline *GATA2* mutation	

Acute myeloid leukaemia (AML) and related precursor neoplasms

AML with recurrent genetic abnormalities

AML with t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*	9896/3
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*	9871/3
Acute promyelocytic leukaemia with *PML-RARA*	9866/3
AML with t(9;11)(p21.3;q23.3); *KMT2A-MLLT3*	9897/3
AML with t(6;9)(p23;q34.1); *DEK-NUP214*	9865/3
AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2, MECOM*	9869/3
AML (megakaryoblastic) with t(1;22)(p13.3;q13.1); *RBM15-MKL1*	9911/3
AML with BCR-ABL1	9912/3*
AML with mutated *NPM1*	9877/3*
AML with biallelic mutation of *CEBPA*	9878/3*
AML with mutated RUNX1	9879/3*

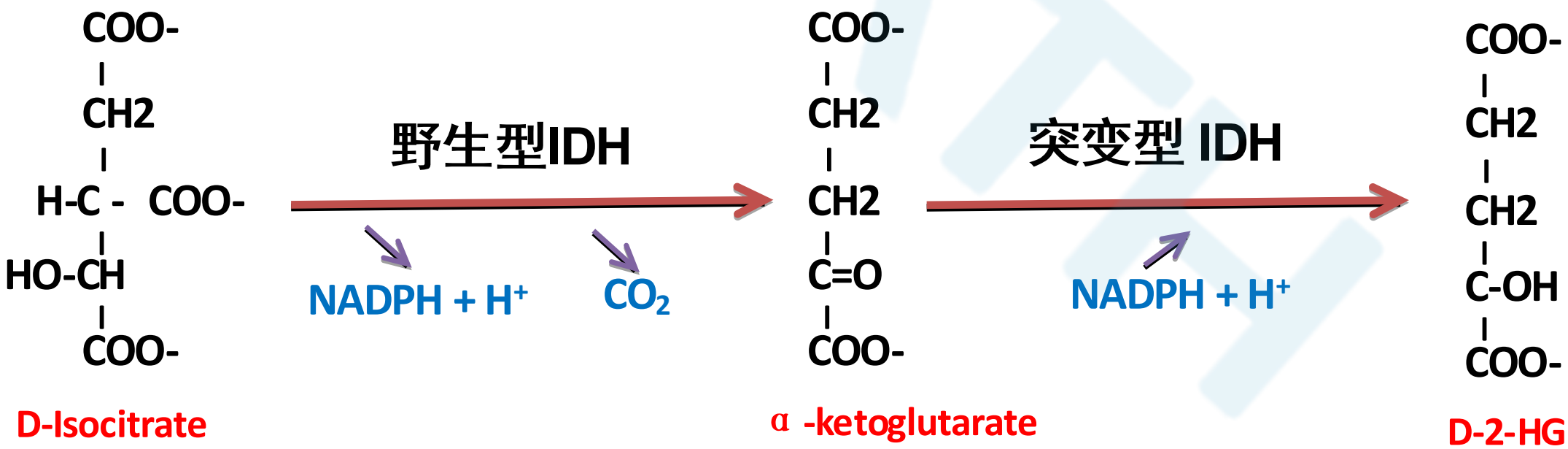
AML with myelodysplasia-related changes	9895/3
Therapy-related myeloid neoplasms	9920/3
Acute myeloid leukaemia, NOS	9861/3
AML with minimal differentiation	9872/3
AML without maturation	9873/3
AML with maturation	9874/3
Acute myelomonocytic leukaemia	9867/3
Acute monoblastic and monocytic leukaemia	9891/3
Pure erythroid leukaemia	9840/3
Acute megakaryoblastic leukaemia	9910/3
Acute basophilic leukaemia	9870/3
Acute panmyelosis with myelofibrosis	9931/3
Myeloid sarcoma	9930/3

Myeloid proliferations associated with Down syndrome

Transient abnormal myelopoiesis associated with Down syndrome	9898/1
Myeloid leukaemia associated with Down syndrome	9898/3

IDH

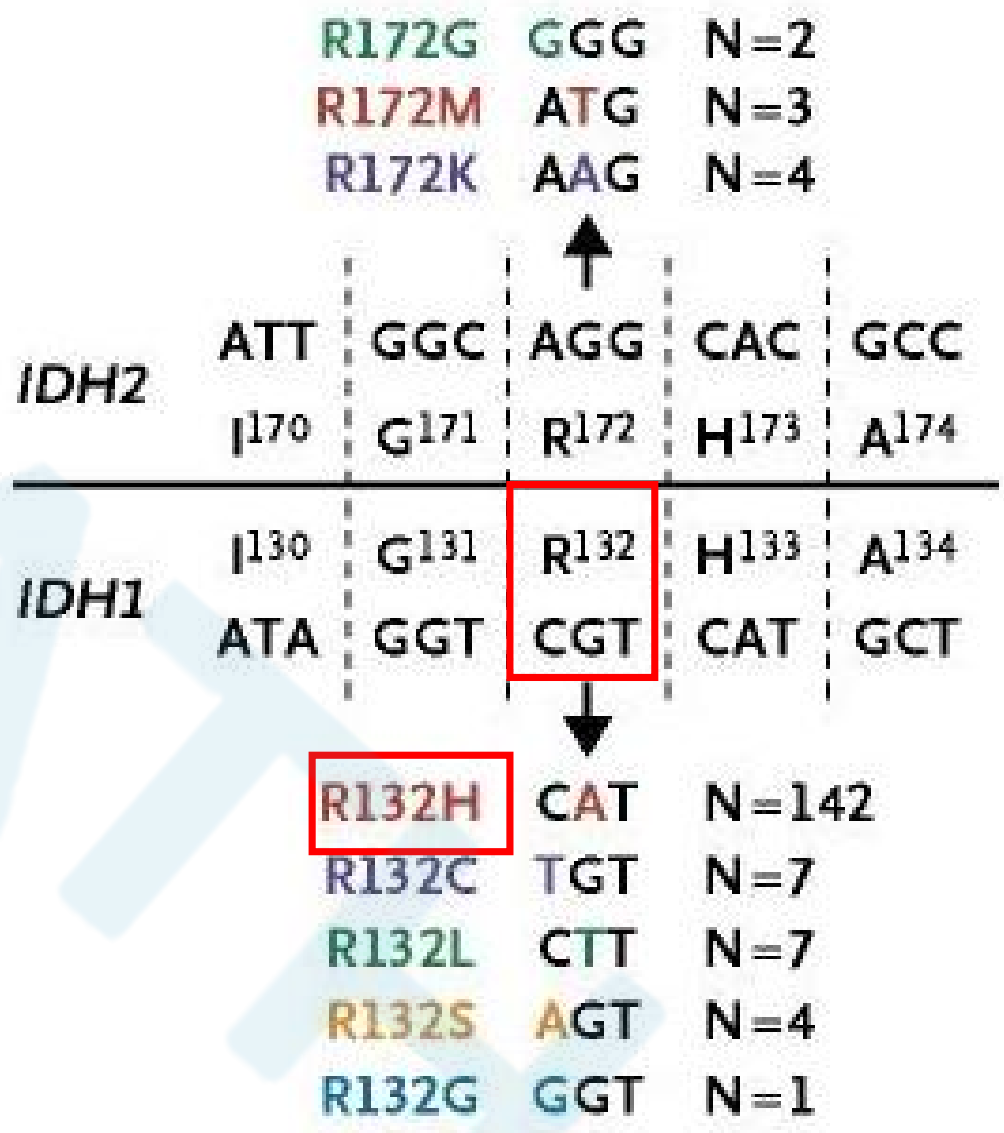
- 异柠檬酸脱氢酶（Isocitrate Dehydrogenase，IDH）
- IDH1在细胞浆；IDH2在线粒体
- **野生型IDH：** 异柠檬酸（Isocitrate）→ α -酮戊二酸（α -KG）
- **突变型IDH：** α -KG→2-羟基戊二酸（2-HG）



参考叶菁教授幻灯

IDH1/2 突变的主要位点

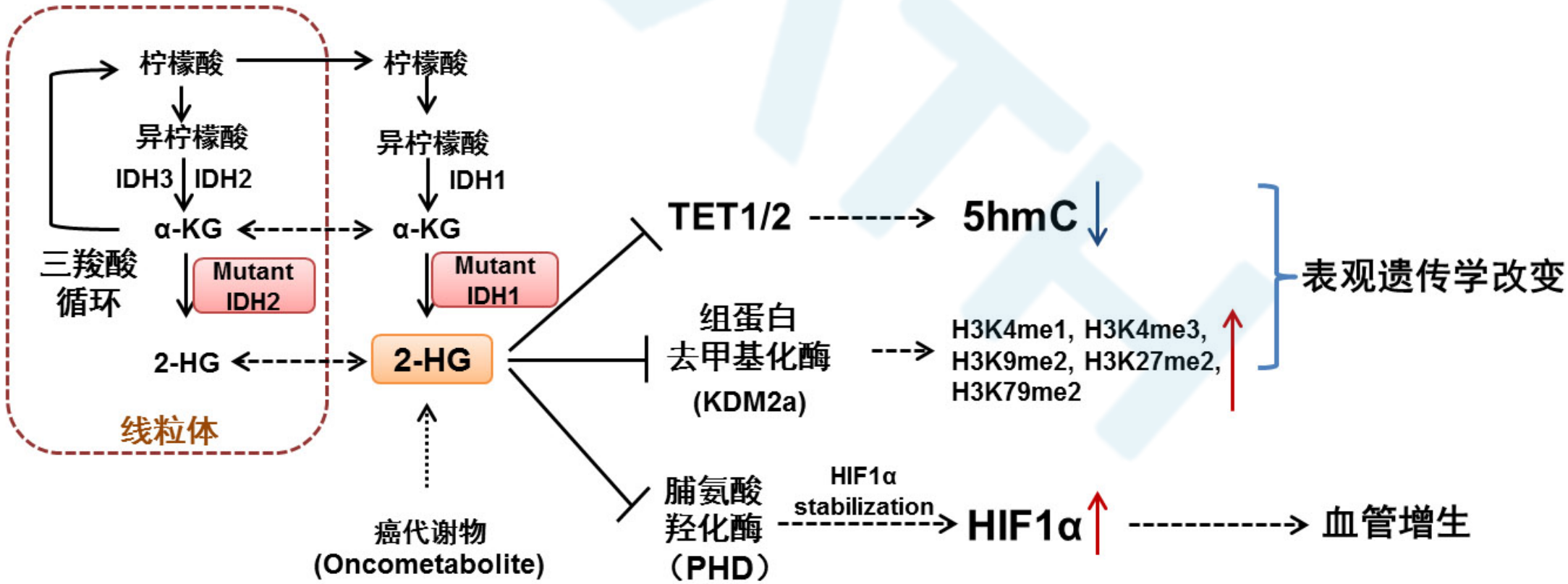
- IDH1/2突变均点突变，且IDH1-R132和IDH2-R172
- IDH1和IDH2突变相互排斥



参考叶菁教授幻灯

IDH突变在肿瘤发生中的作用

- 尚未明确
- 2-HG（癌代谢物），能竞争性阻断α-KG依赖的反应
- 最近研究发现，IDH1/2突变影响代谢过程



参考叶菁教授幻灯

Objective

- 在胶质瘤和血液恶性肿瘤中已经鉴定出IDH1 / 2突变。虽然IDH突变在原发性AML中的预后意义存在争议，但强大的数据显示IDH突变有助于MDS和MPN的白血病转化
- 并且，IDH突变还经常与其他众所周知的MDS，MPN和AML的基因突变（如DNMT3A和RUNX1）共同发生
- 另外，IDH1/2突变的靶向抑制剂目前正在临床试验中进行评估，为了促进这些靶向药物的使用，快速、低成本、敏感和特异性的筛查技术的可用性是非常重要的
- 所以，作者对IDH1 p.R132H突变特异性抗体在血液肿瘤中的应用进行了研究

Case Selection

TABLE 1. Distribution and Molecular Characterization of Cases

All Patients (Np = 93, Ns = 143)

I. WT *IDH1* (Np = 10, Ns = 11) all AML patients

II. Non-R132H (Np = 27, Ns = 30) all AML patients

R132C (Ns = 17)	R132S (Ns = 3)	R132G (Ns = 2)	R132L (Ns = 6)	R132S, R132C (Ns = 1)	R132C, R132G (Ns = 1)

III. R132H (Np = 56, Ns = 102)

	Initial				Follow-up			
			MAF (%)				MAF (%)	
	Np = 49	Ns = 49	Range	Median	Np = 29	Ns = 53	Range	Median
AML-NOS	17	17	4.8-47.2	24.05	15	31	1.5-46.5	19.95
AML-MRC	11	11	1.8-46.7	17.65	7	11	3.1-43.7	23.8
t-AML	1	1	30.1	30.1	0	0	NA	NA
MPN-BP	1	1	42.5	42.5	1	4	7.2-36	26.5
MDS	10	10	2.3-44.3	16.35	2	2	0	0
MDS/MPN	4	4	4.3-47.2	23.9	2	2	0	0
MPN	5	5	3.7-43.9	23.85	2	3	21.5-36.1	36

NA indicates not applicable; Np, number of patients; Ns, number of specimens; t-AML, therapy-related AML.

持续性疾病 **Ns=71**

流式检测**MRD** **Ns=9**

流式及形态学完全缓解 **Ns=13**

完全缓解后复发病例 **Ns=9**

IHC and Molecular Testing

TABLE 2. Comparison of IDH1 p.R132H IHC and Molecular Studies in Primary and Follow-up Cases

	Initial	
	Molecular (+)	**Molecular (−)**
IHC (+)	49	0
IHC (−)	0	41

IHC indicates IDH1 p.R132H IHC; molecular, *IDH1* p.132H mutation

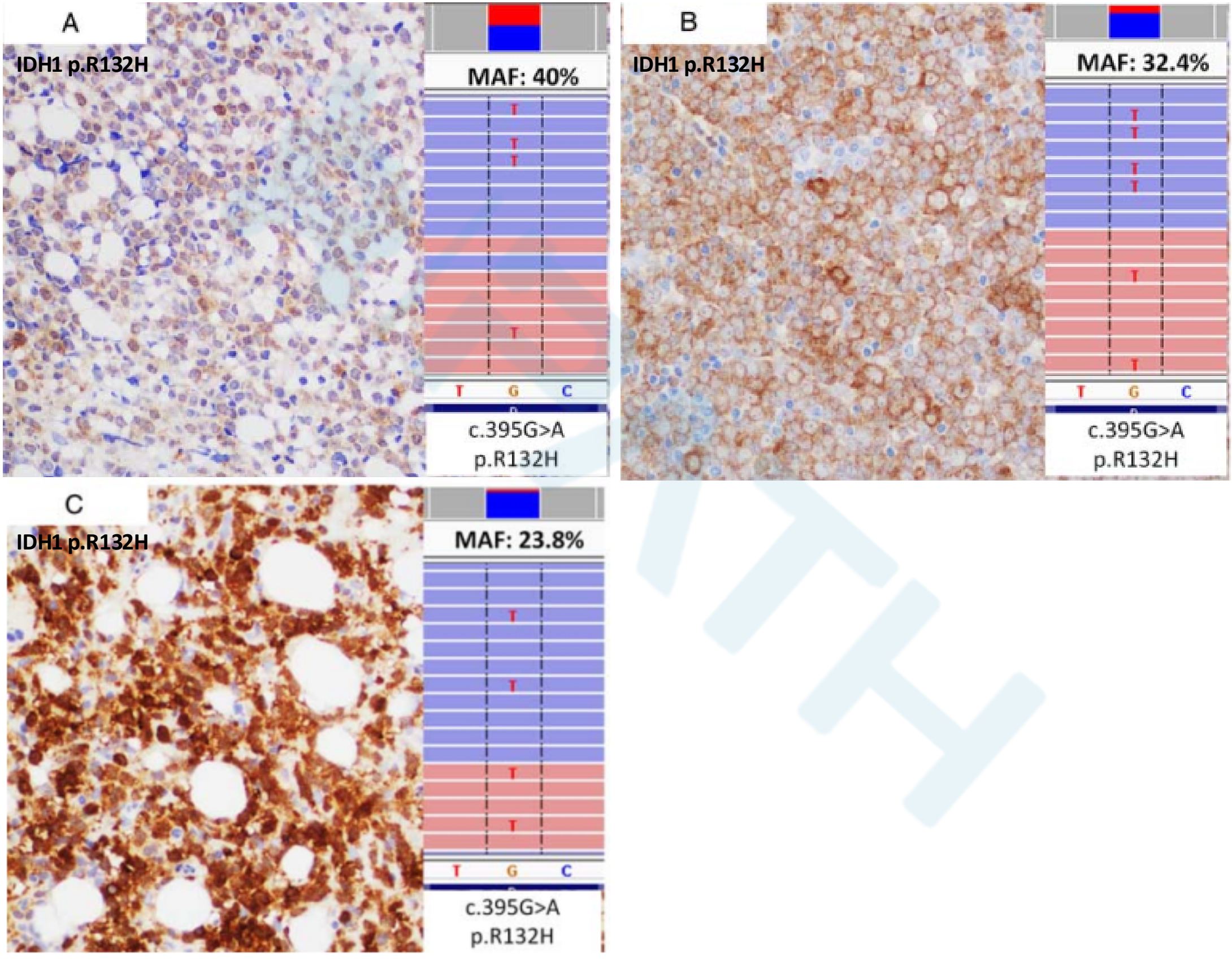
AML (n = 30)

MDS (n = 10)

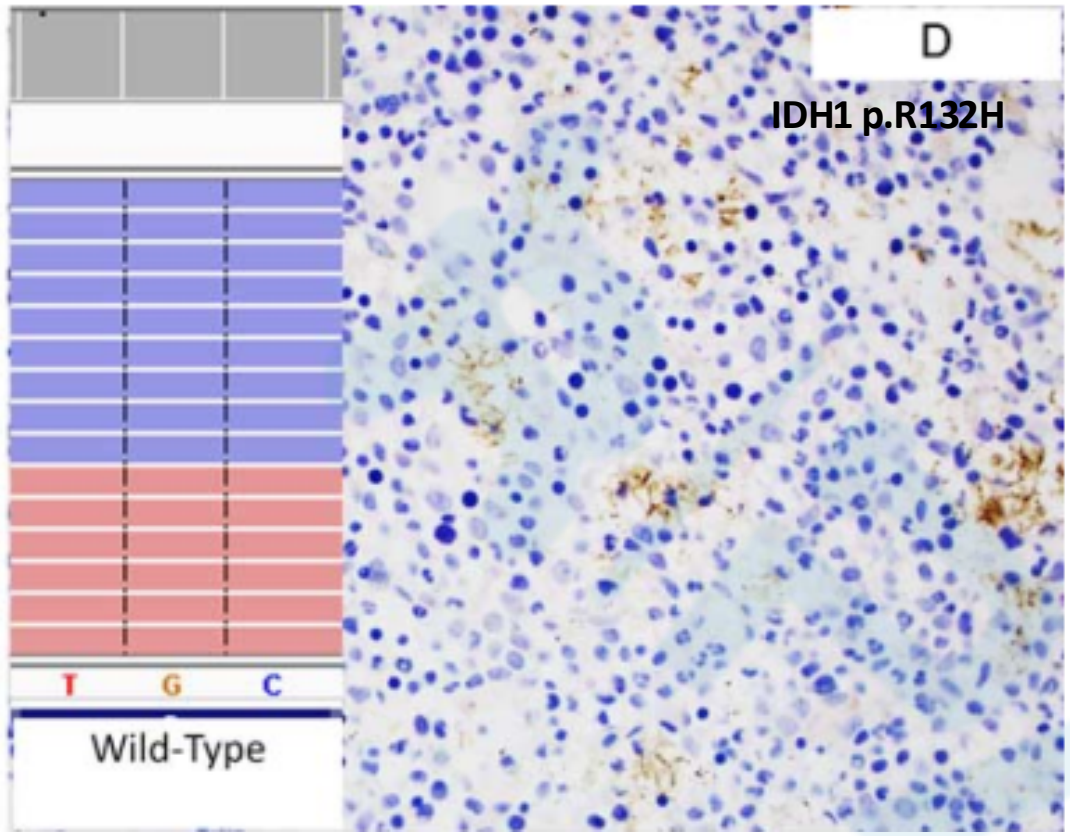
MDS/MPN (n = 4)

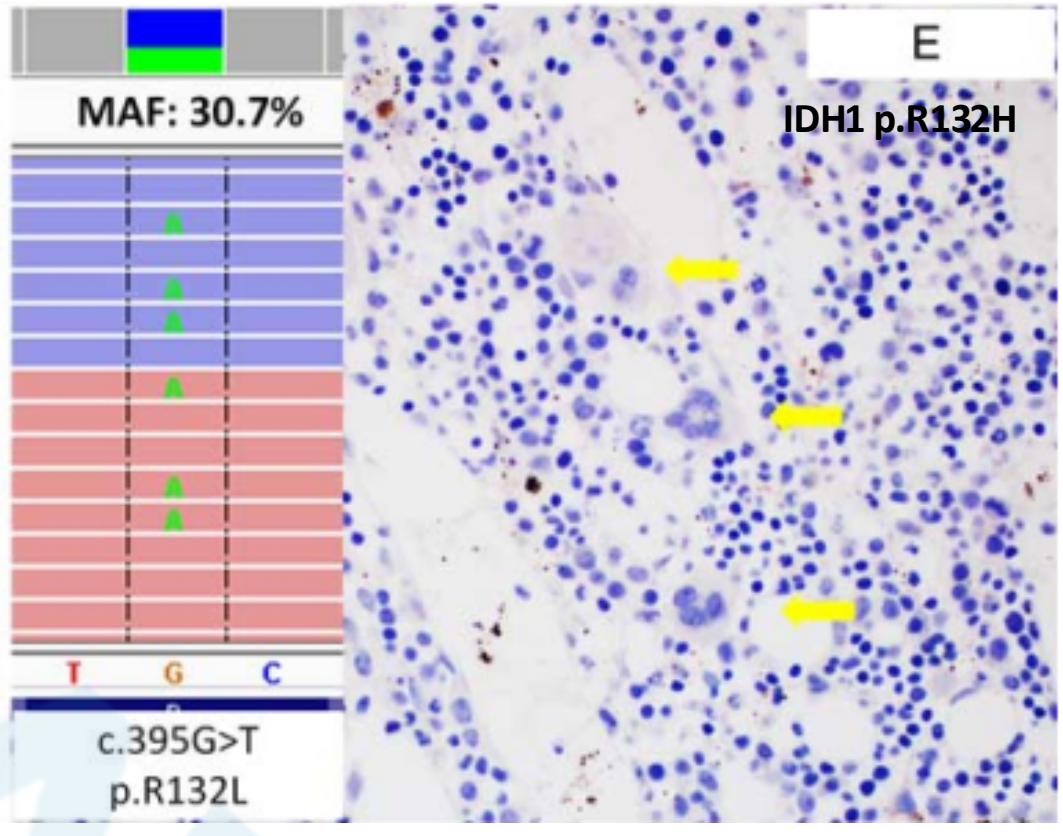
MPN (n = 5)

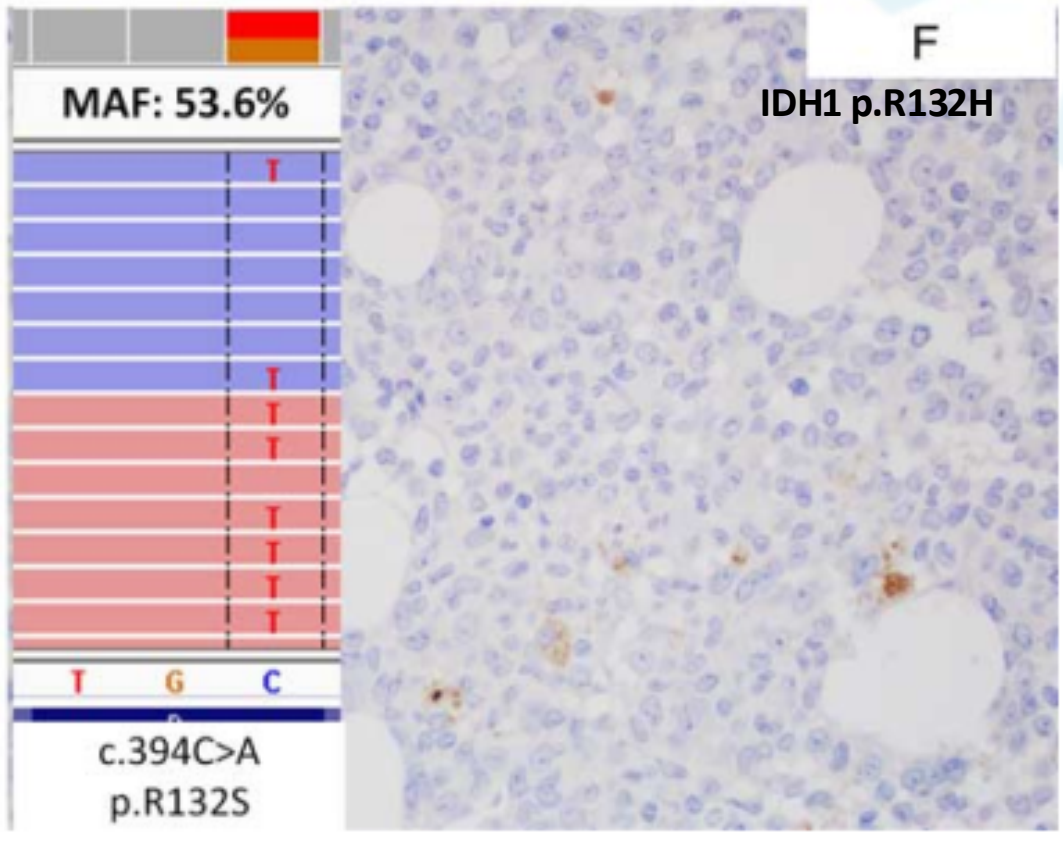
Performance of IDH1 p.R132H Immunostaining in Primary Patient Samples



Performance of IDH1 p.R132H Immunostaining in Primary Patient Samples







IHC and Molecular Testing

TABLE 2. Comparison of IDH1 p.R132H IHC and Molecular Studies in Primary and Follow-up Cases

			Follow-up	
	AML (n = 23)	Np = 29		
	MDS (n = 2)	Ns = 53	Molecular (+)	Molecular (−)
	MDS/MPN (n = 2)	NGS (n = 46)		
IHC (+)	MPN (n = 2)	Sanger (n =7)	33	8
IHC (−)			0	12

IHC indicates IDH1 p.R132H IHC; molecular, *IDH1* p.132H mutation

NGS (n = 5)

Sanger (n =3)

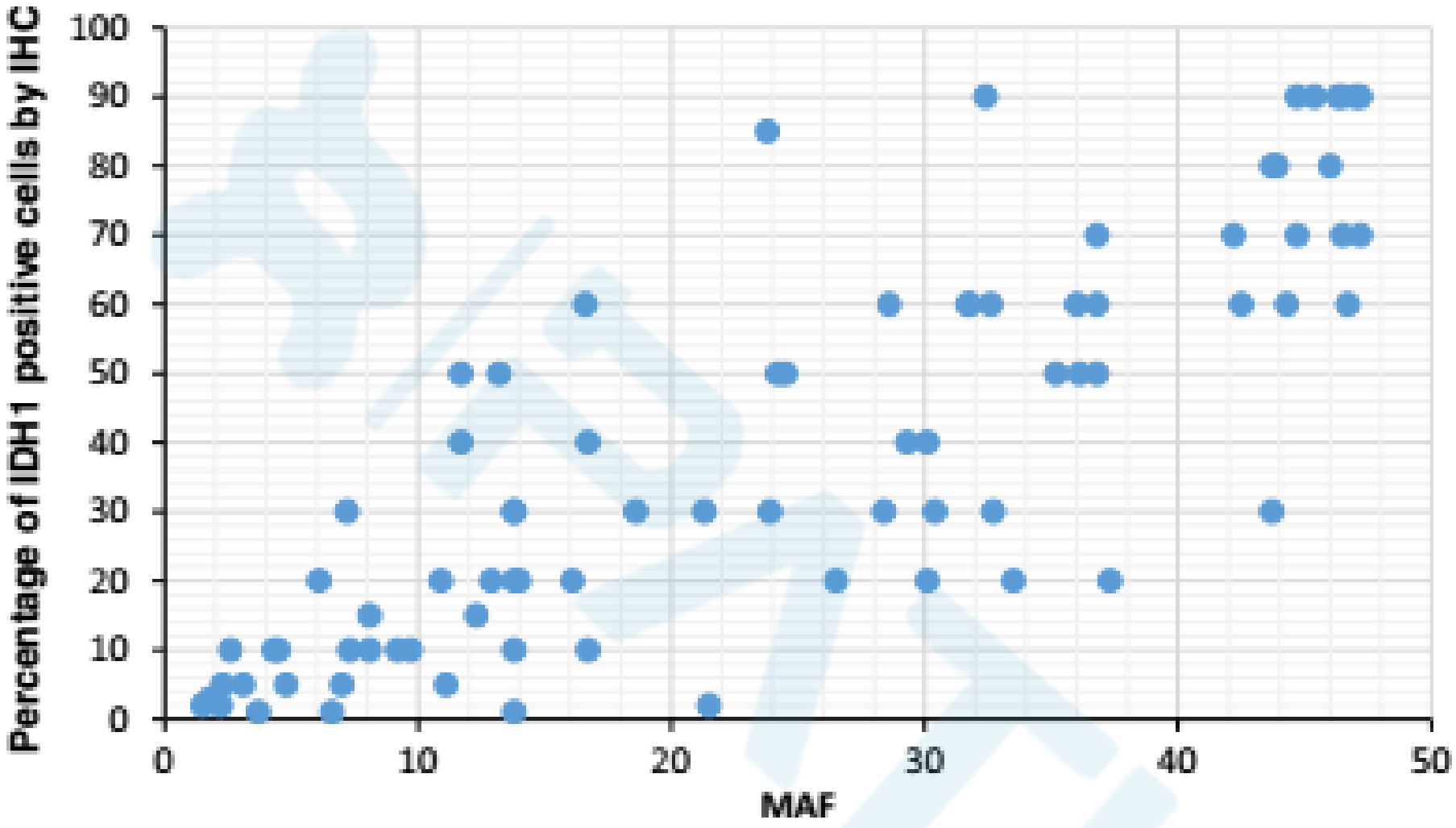
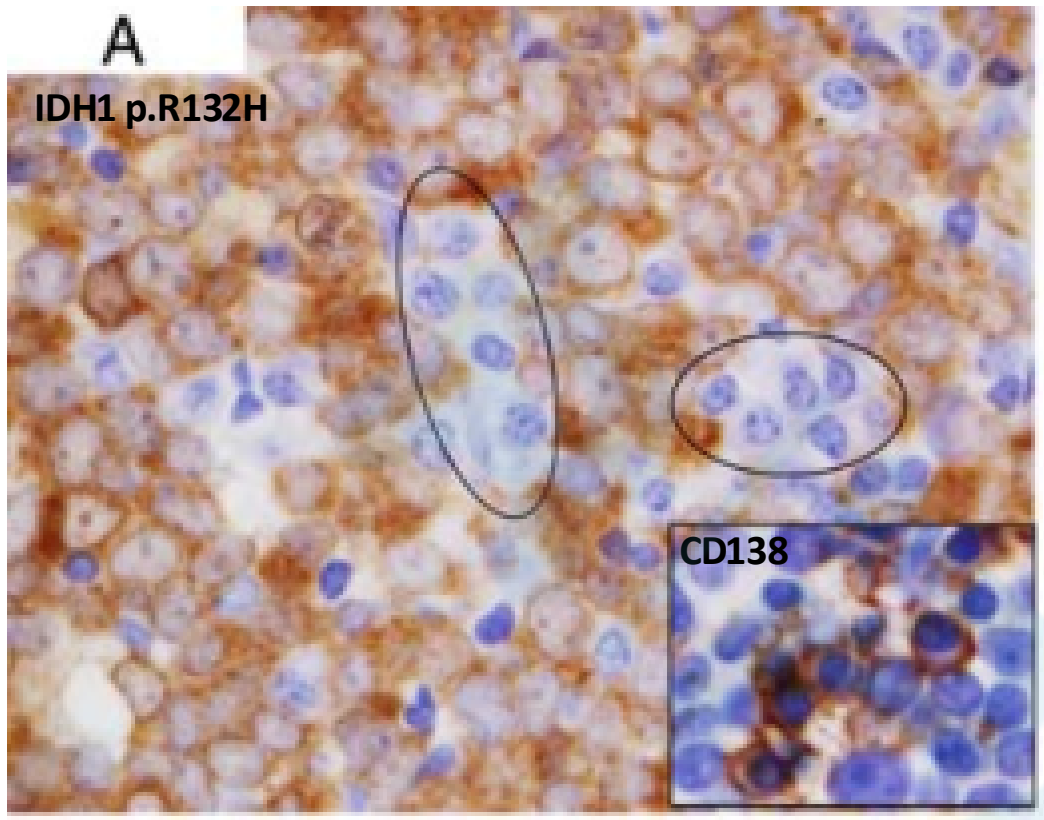
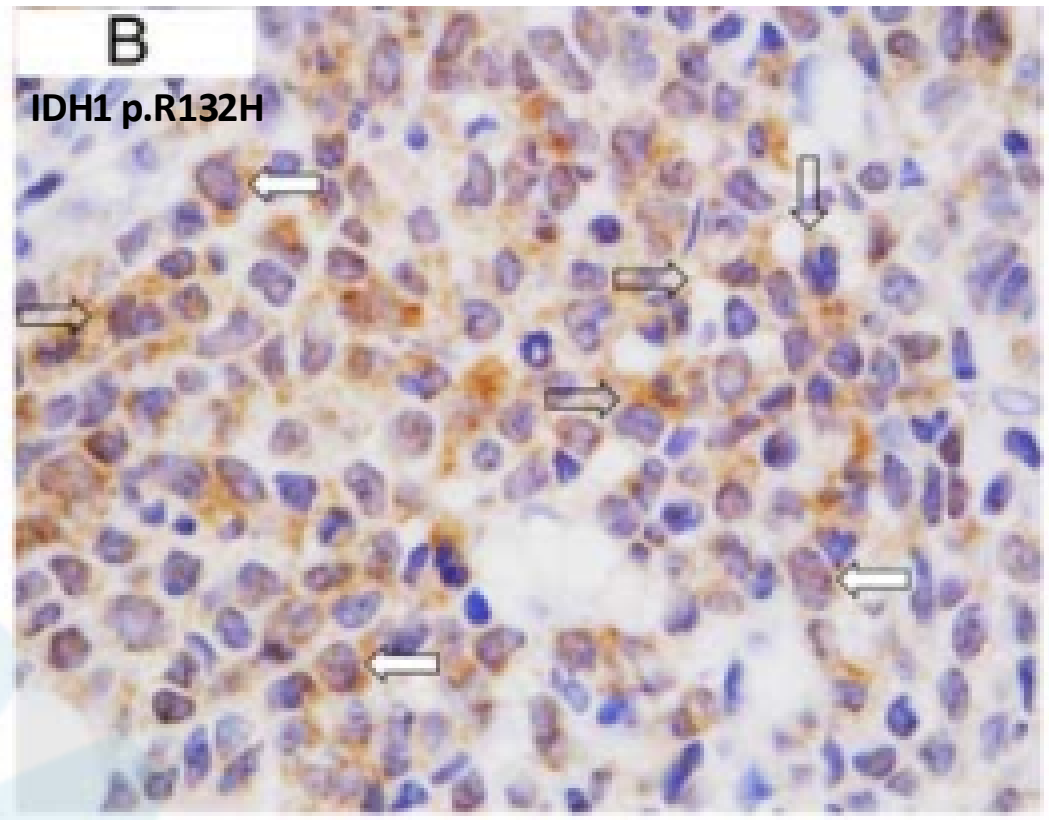
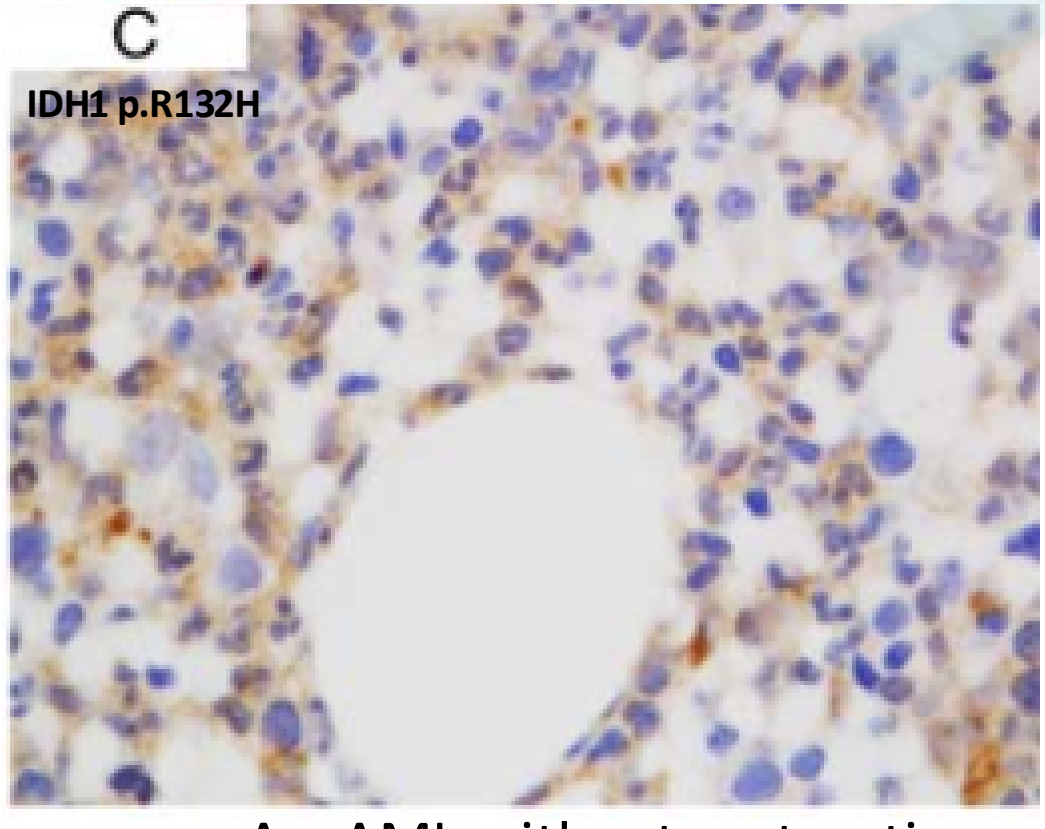


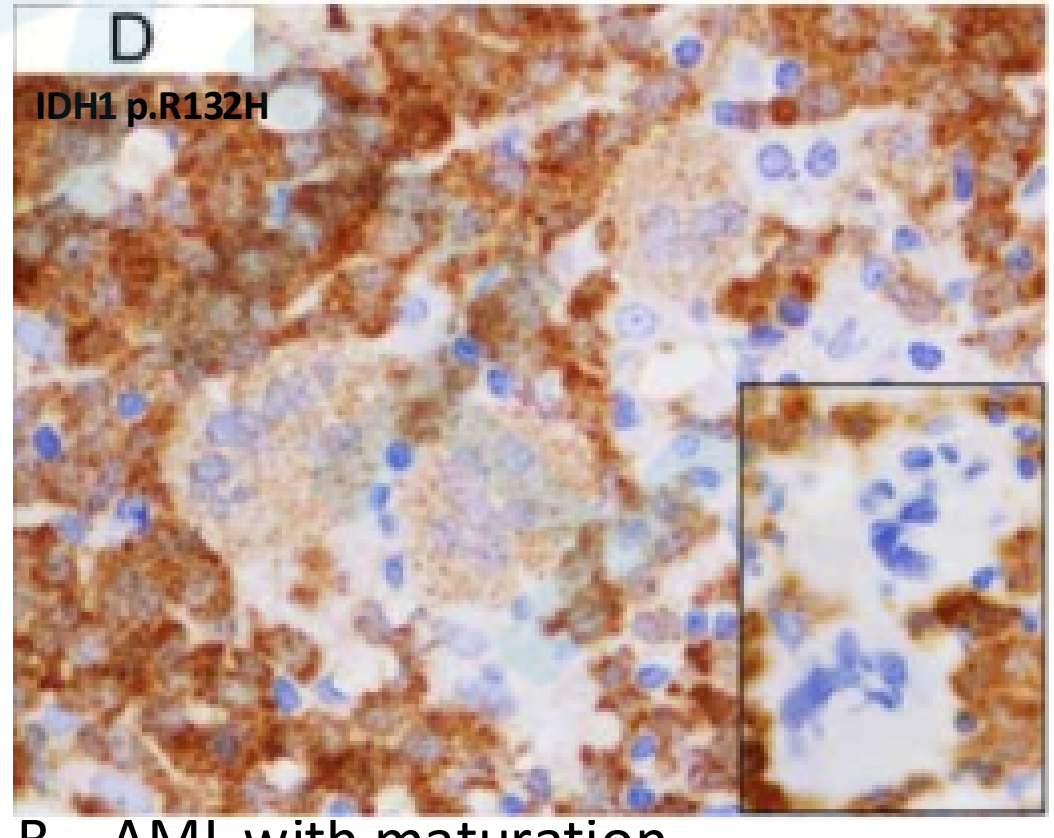
FIGURE 3. Comparison of MAF and percentage of positive cells by IDH1 p.R132H IHC.

Morphologic Characterization of Mutant Clones



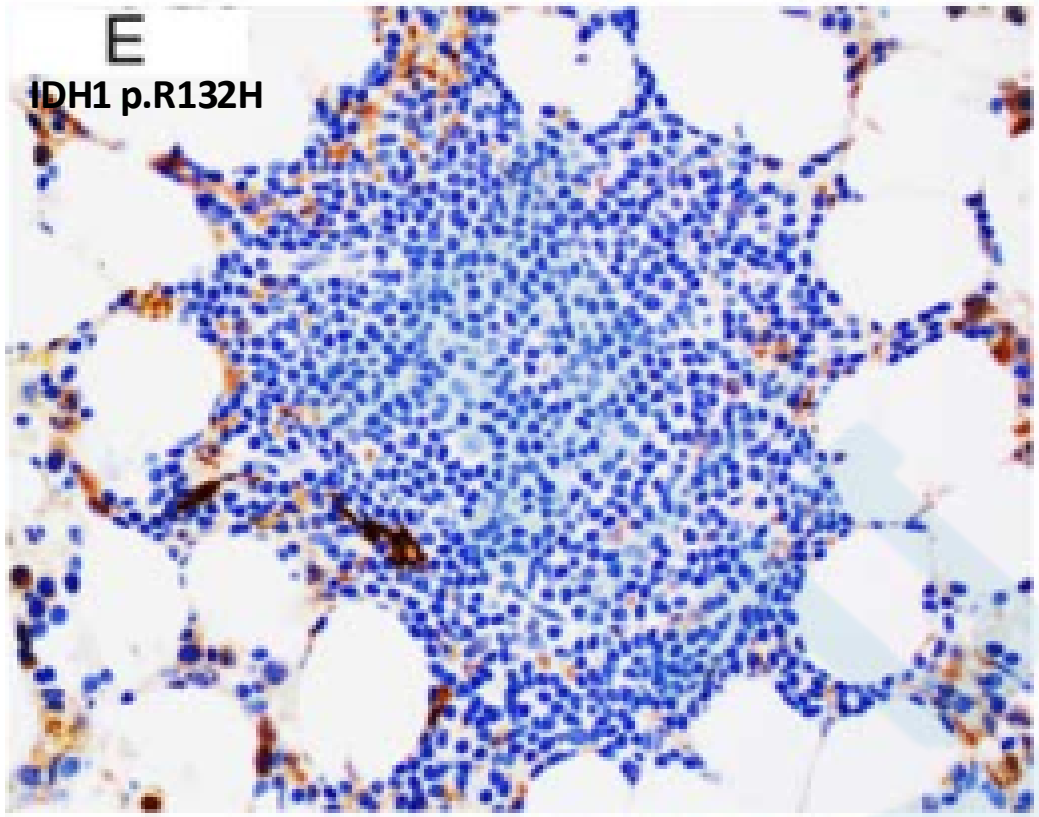


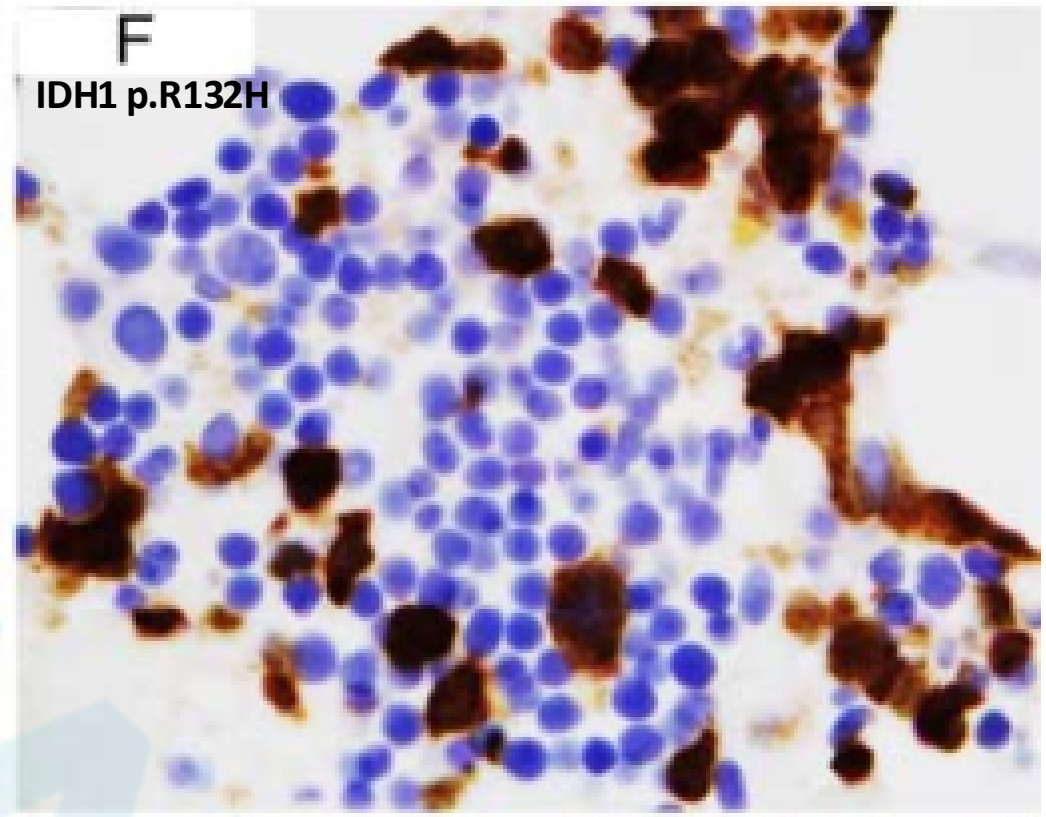


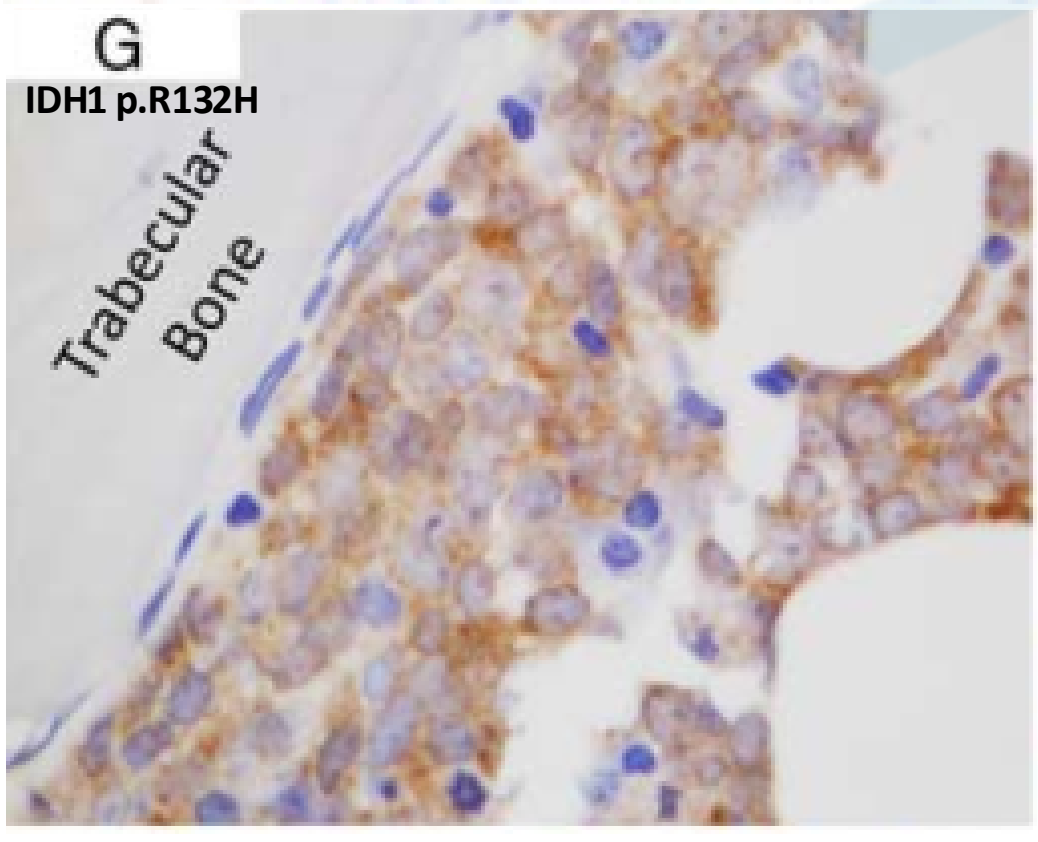


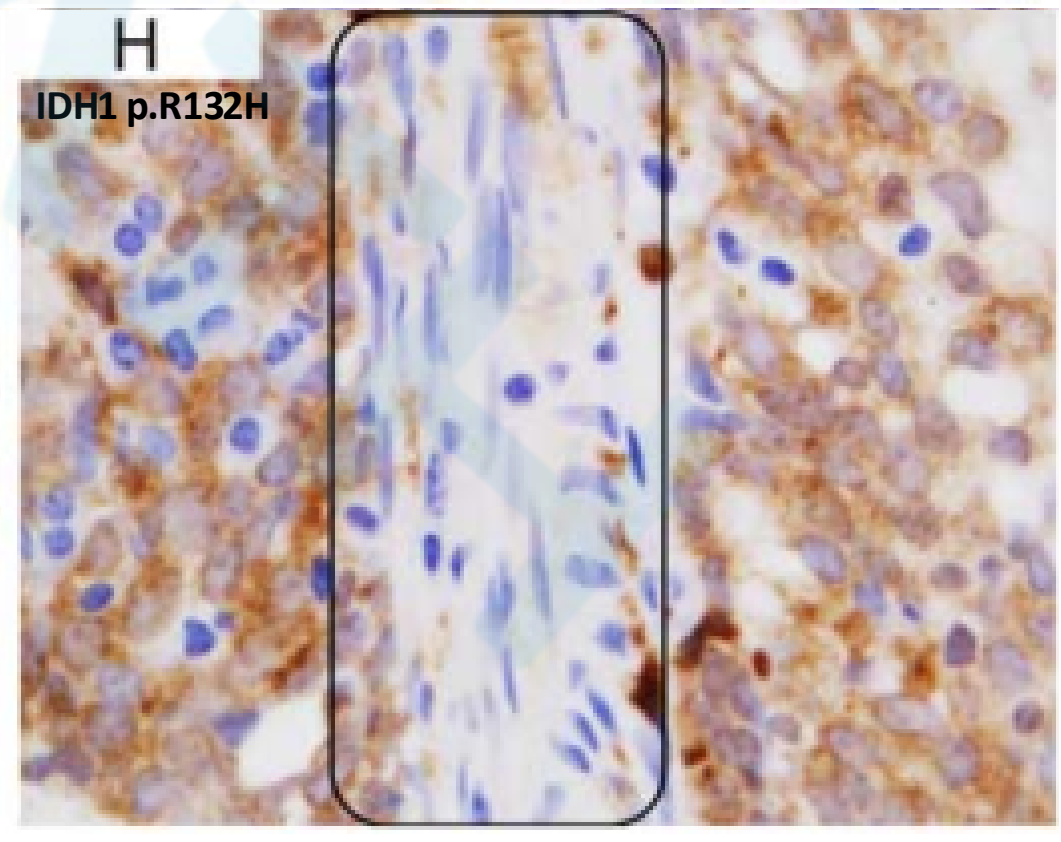
A. AML without maturation
B. AML with maturation
C. MDS/MPN
D. AML with minimal differentiation

Morphologic Characterization of Mutant Clones

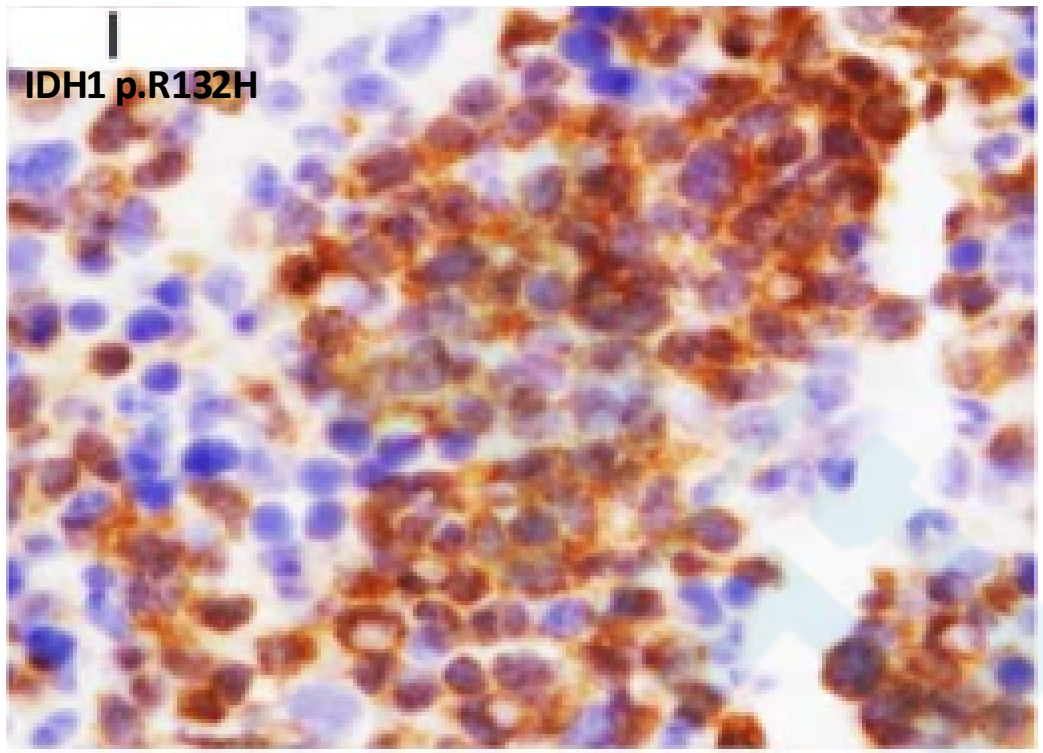


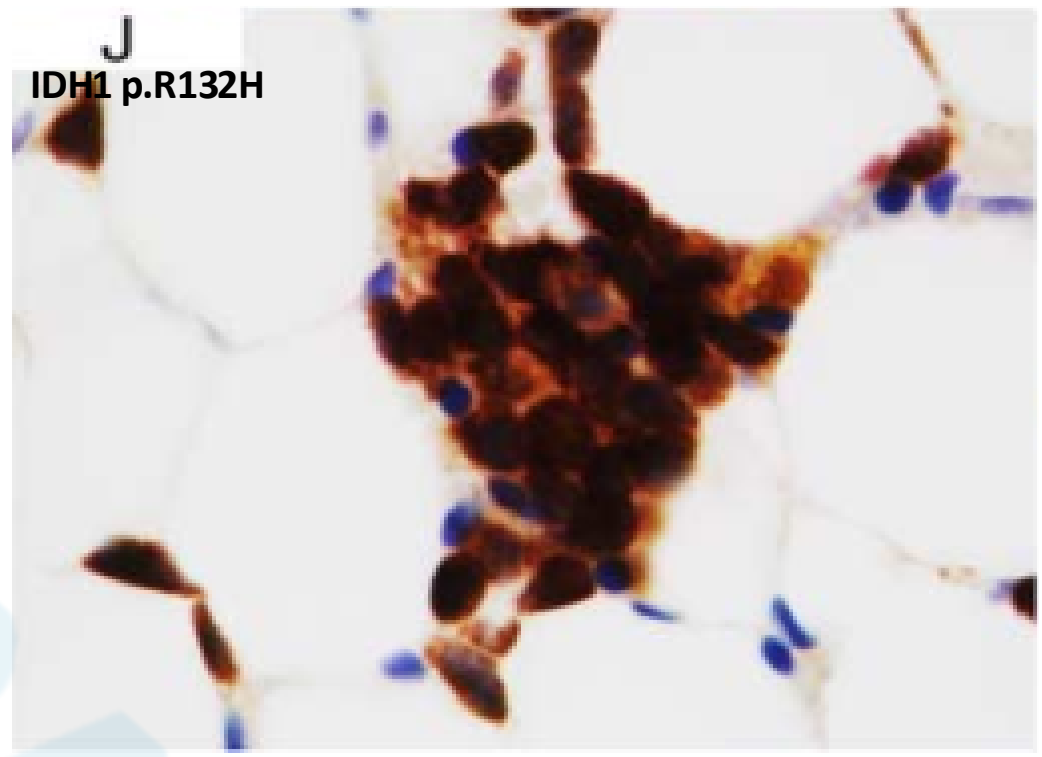


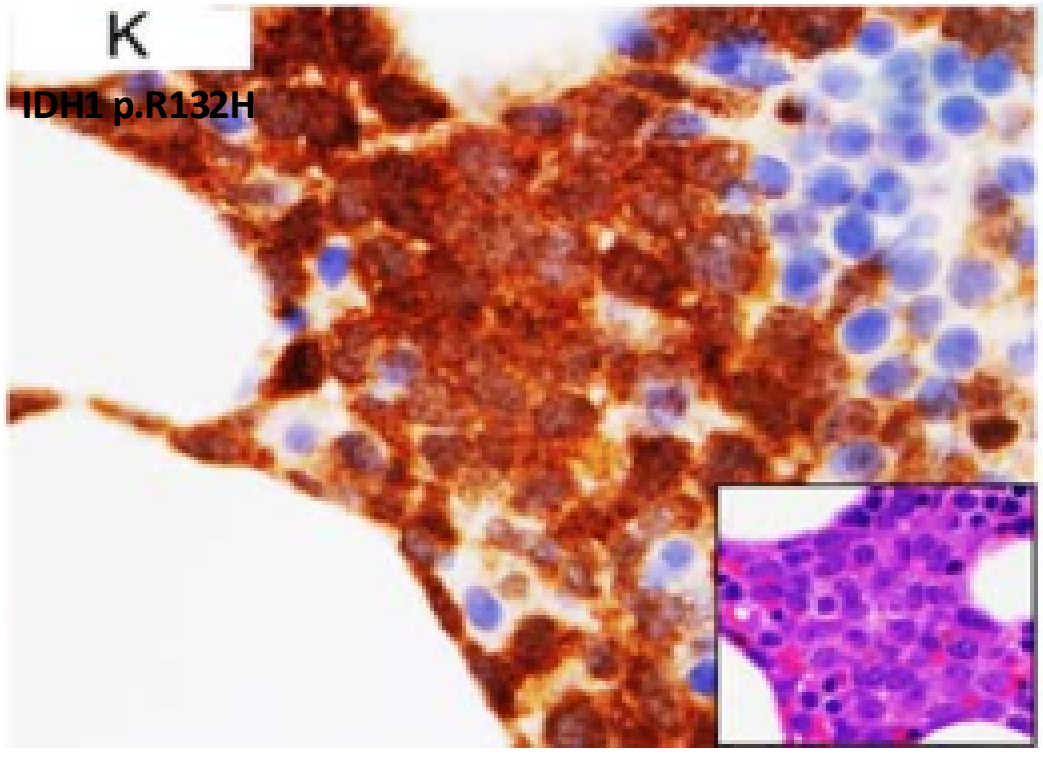


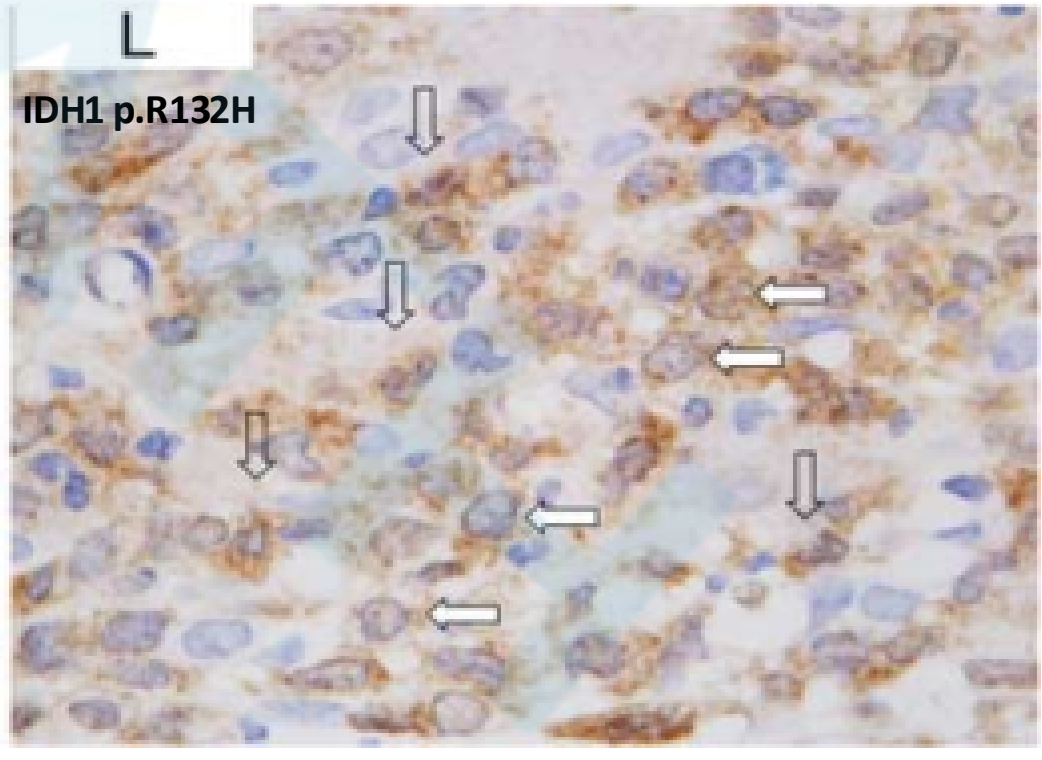


Morphologic Characterization of Mutant Clones









I. AML with MRC J. Posttreatment persistent AML
K. Relapsed AML L. Posttreatment persistent AML

Discussion

- 本研究显示IDH1 p.R132H的IHC是一种高度特异性和敏感的方法，与分子检测具有较高的一致性（85%）。
- 其中，不一致率主要见于分子检测阴性，但IHC仍为阳性，可能由于低肿瘤负荷、采样问题和分子检测的限制性。
- IHC显示骨髓单核细胞和巨核细胞胞浆阳性，而且大多数未成熟细胞也是阳性，而红系前体细胞、淋巴细胞、内皮细胞、骨母细胞为阴性，这表明IDH1突变是体细胞突变，而不是胚系突变。
- 在胶质瘤中，IDH1/2突变已被证明与更好的预后相关，然而， IDH1/2突变在血液肿瘤中的预后尚未明确。
- 虽然IDH突变在原发性AML中的预后意义存在争议，但强大的数据显示IDH突变有助于MDS和MPN的白血病转化。

Discussion

- 其他文献中的小鼠模型显示， IDH突变足以通过组蛋白高甲基化、DNA甲基化改变和部分阻断髓系细胞分化启动血液恶性肿瘤。
- 然而，这些模型中肿瘤的长潜伏期和不完全外显率表明，需要白血病转化的二次突变。
- 本文中，有1例有MDS转化为AML的病例，在MDS标本中，大多数IHC阳性细胞为成熟细胞，而在白血病转化的标本中，成熟细胞和未成熟细胞均阳性。
- 这进一步表明，IDH突变使白血病转化需要二次突变。

Discussion

- 作者还评估了 IDH1 p.R132H IHC 在 MRD 检测中的价值
- 本研究首次证明，成熟髓样细胞是治疗后形态学和免疫表型完全缓解的病例中 IDH1 突变的来源
- 这一重要发现可以提高我们对分子 MRD 在临床应用的理解
- 鉴定 IDH1 突变阳性细胞的成熟度还可以更好的了解每种疾病的生物学特征，选择可以从 IDH1 突变抑制剂治疗获益的病例，并评估治疗效果
- 本文观察到在不同病例中 IHC 表达强度不同，这表明在蛋白表达水平上可能调节 IDH1 活性
- 所以，不能将 IHC 信号强度与疾病预后相关联，应该研究突变蛋白的表达水平，它可能与预后相关

Conclusion

- IDH1 p.R132H 特异性抗体是一种高度特异和敏感的抗体，用于检测各种髓系肿瘤中的突变克隆并对其进行可视化
- IHC 可以表征阳性细胞群，更好地理解疾病的生物学特性，并有助于评估每位患者的治疗反应

hank You for your attentio