Keywords: O6-me h lg anine-DNA me h l an fe a e; Gliobla oma; Real- ime ol me e chain eac ion; Temo olomide; C o oin ; Be aci mab; Recei e o e a ing cha ac e i ic anal i

Introduction

Temo olomide (TMZ) i he onl chemo he a e ic agen hich ha he le el 1 e idence fo he gliobla oma (GB) ea men . Among all olid mo incl ding b ain mo , i i adj dged o elici o ogno i [1,2]. Indeed ome o he an i- mo d g ch a 1-(4-amino-2-me h l-5- imid n l) me h l-3-(2-chlo oe h l)-3-ni o o ea h d ochlo ide (ACNU), Be aci mab, H man in e fe on- , Vinc i ine, and Ca m ine afe a e a o ed and ed fo GB he a in Ja an, b he e eem o be e li le e idence i h ega d o hei e ec i ene fo GB he a [3]. Addi ionall , e ce fo Be aci mab, he e ha e been no e o on he e ec i ene of he e d g oge he i h TMZ. e e ec of Be aci mab in addi ion

Ho i al, Tok o, 10 a ien a Na ional Cance Cen e Ho i al, Tok o; 1 a ien a Shio a Ho i al, In e na ional Uni e i of Heal h and Welfa e, Yai a; 3 a ien a Ka a aki Ho i al, Hi achioh a; and emaining 1 a ien a Tok o-Ni hi Tok h kai Ho i al, Tok o, Ja an. All of he 55 GB a ien ecei ed emo olomide and adia ion a e ge in acco dance i h he S o ocol [2]. W i en info med con en fo he an i a ion of MGMT mRNA in mo am le a o ided b all a ien . RT-PCR ba ed an i a ion of MGMT mRNA (C o Poin of MGMT mRNA in Gliobla oma) a a o ed b he E hic Commi ee a Tok o Medical Uni e i in he ea 2005, a Ki a a o Uni e i in he ea 2002, a he In e na ional Uni e i of Heal h and Welfa e in he ea 2012, and a Tok h kai Ho i al in he ea 2015.

Real-time polymerase chain reaction based quantitation of MGMT mRNA absolute value

Collec ion of mo am le and an i a ion of MGMT mRNA a e fo med b S ecial Refe ence Labo a o Co. L d., Hino, Ja an. e me hod ed o an if he ab ol e al e of MGMT mRNA b RT-PCR a de c ibed e io l [9]. B ie , he g anidini m hioc ana e- henol-chlo ofo m media ed e ac ion a e fo med ing I ogen (WAKO J n ak) fo e ac ing o al RNA f om ei he 10 mg of f e hl ob ained mo am le o ed a 4 C in QIAGEN RNAla e Ti e P o ec T be (AMBION Inc) o i e f o en a -70 C [10]. F om 1 g of he e ac ed o al RNA, he com lemen a DNA (cDNA) a n he i ed and a b e en l inc ba ed a 37 C fo 60 min e . e eal- ime ol me a e chain eac ion a ca ied o ing a Ta Man Uni e al Ma e Mi (A lied Bio em) com i ing of 120 nM of each ime [11], 200 nM of obe (5-CGA GCA GTG GGA GGA GCA ATG AGA-3), and 2.5 L of each cDNA am le, i h dena a ion a 95 C fo 10 min e and 50 c cle (a 95 C fo 30 econd , 60 C fo 40 econd , and 72 C fo 30 econd) in a eal- ime PCR em. e le el of gl ce aldeh de-3-ho ha e deh d ogena e (GAPDH) mRNA e e ion e e ed a a an i a i e in e nal con ol. U ing a anda d c e, he e e ion le el of each mRNA a calc la ed. In o de o ob ain an e en mo e acc a e an i ca ion, he MGMT mRNA e e ion le el of each am le a no mali ed b he e e ion of he GAPDH gene.

Statistical analysis

All he a i ical anal e e e ca ied in Mic o o E cel Tokei So a e. e og e ion-f ee i al e iod and o e all i al of he 55 GB a ien e e anal ed, and he ca e i h le han 8.1 mon h og e ion-f ee i al and 15 mon h o e all i al e e j dged o be TMZ e i an acco ding o he e l of B ain T mo Regi Ja an [12]. e c o oin e e de e mined in GB ea ed i h TMZ le han 75 and KPS of a lea 60 b ROC anal i . e eci ci and en i i i of each c o oin e e calc la ed. Ka lan-Meie anal i a e fo med o e al a e each i al ime, and he log ank (Man el-Co) e a con ide ed fo anal ing he bina a iable (le han and a lea he c o oin). 2- ailed p al e a e e o ed. De e mina ion of he a i ical igni cance of he da a anal i a e a a obabili le el of 5% (p=0.05).

Results

ROC analysis for selecting candidate cutoff points for MGMTmRNA in GB

e candida e c o oin in each GB g o e e calc la ed b aco ie /0371gRNA fe e che ca dida e c o oin io a e di ing PFS

ac dida e c o oin io a e di ing OS eci ci ie of a83.6, 7.9()Tj0.208 T TKacd K69.6%,and en i i i ie of a39.1, 47.8and 502.2%,ae iecii

o]TJ0.077 T TKj im e c].65aach i al 0.5(ome, and he)og ank .5(Man el-Co) 0.5(o e e d]TJ0.10 T TK ed ao e al a e eina

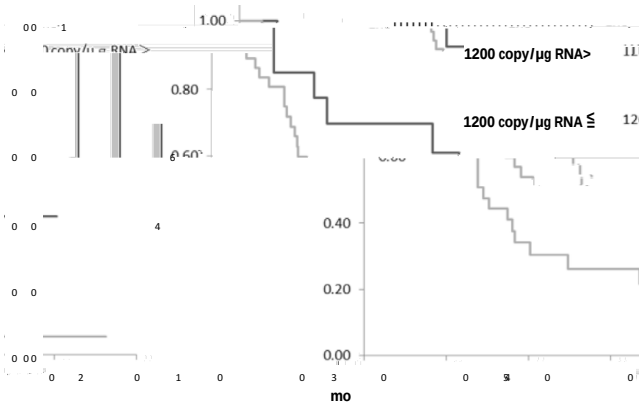
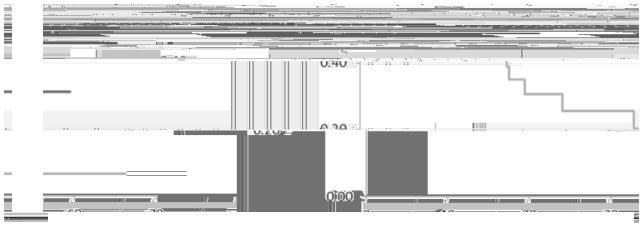


Figure 3:

and radiation for GB with less than and at least 1200 copies of MGMT mRNA/µg RNA for overall survival (OS). OS was signi f cantly longer in those who had less than 1200 copies/µg RNA (P=0.0189 by logrank test).



Discussion

A men ioned in he in od c ion ec ion, MGMT i ilenced b h e me h la ion of DNA omo e domain [6]. Man e of MGMT, ch a imm nohi ochemi , me h la ion- eci c PCR, o e encing, So he n blo ing, and he ab ol e al e of MGMT mRNA, ha e been e o ed [13]. Among he e me hod , imm nohi ochemi and me h la ion- eci c PCR a e no f ndamen all an i a i e, and he efo e he a e ina o ia e fo a ce aining he indica ion and oced e fo TMZ he a , al ho gh Hegi e al. [7], b an ia e he edic i e o en ial of a an i a i e me h la ion- eci c PCR fo he e al a ion of MGMT omo e me h la ion. Imm nohi ochemi ield lo eci ci hile i i no an i a i e a e he anal i of MGMT a he o ein le el [14]. e ac i i of MGMT a ea o ha e high co ela ion i h clinical e i ance o alk la ing agen ho gh mRNA le el a an i a ed b RT-PCR migh no co ela e i h en ma ic ac i i al a [15]. mRNA e e ion ha been efe en iall in e iga ed beca e an i a ion of MGMT ac i i i e com le e i ing longe ime fo clinical ili [11,16]. Real- ime ol me a e chain eac ion echni e i ela i el im le, ick and clinicall ele an fo he e ima ing he e en of e i ance o he alk la ing agen a de c ibed e io l . In a e io d , he e l e aining o he me h la ion eci c PCR and e encing e e com a ed o he MGMT mRNA e e ion le el ba ed on eal- ime an i a i e RT-PCR and ha e e o ed ha he e en of MGMT mRNA e e ion a ongl co ela ed i h he me h la ion a of MGMT omo e [17]. Hence, he le el of MGMT mRNA e e ion a ea o be ela i el mo e eci e ogno ic fac o han he e l f om me h la ion- eci c PCR.

In fac , ecen l o e encing a e ec i el ed fo he o e of an i a i e de ec ion of me h la ion. e e en of MGMT me h la ion ba ed on o e encing a e io l e o ed a fac o of ogno i in ca e of GB he a b emo olomide a ell a adia ion, b clea c o oin fo a licabili of o e encing o e ima e MGMT me h la ion a no a ce ained [18]. Se e al e o on c o oin of me h la ion of MGMT omo e ba ed on o e encing and he eci e elec ion of ho o of omo e me h la ion im ac he e l [19,20]. On he con a , ba ed on SYBR G een me hod, E e ha d e al. e o ed a me h la ion a conco dance le el of a high a 85% be een o e encing and RNA e e ion le el b

RT-PCR and f he ecommended ha if e e ion of an c i ion i he ke mechani m fo highe chemo en i i i of mo i h MGMT me h la ion, hen a b an ial a e of di co dance a an ca ion hile deciding he a e ic a eg olel ba ed on MGMT me h la ion a [21].

On he o he hand, almo all e io clinical die ha e e cl ded elde l a ien and ho e i h a lo KPS. Age and KPS ha e been e o ed o be he mo igni can ogno ic fac o in GB [19]. An im o an objec i e of he e en d a o iden if he c o oin fo MGMT mRNA ab ol e al e fo adding o he an i- mo agen o TMZ and adia ion he a . e e en d ho ed ha , in GB a ien ea ed i h TMZ and adia ion ho e e le han 75 and ho had a KPS of a lea 60, 1200, 2100, 2900 and 3600 co ie / g RNA of MGMTmRNA e e he candida e c o oin fo edic ing bo h PFS and OS. Among he e, igni can l longe PFS and OS e e ob e ed in a ien ho did no e ceed 1200 co ie / g RNA. One o and and o h nd ed co ie / g RNA a ea ed o be he mo ea onable c o oin of MGMTmRNA in GB fo deciding o e o he an i- mo d g ch a Be aci mab oge he i h TMZ. GB a lea 1200 co ie / g RNA eemed o be able o ole a e TMZ, o he an i- mo d g ma be ed i ho TMZ. e fac ha MGMT i a icide en me and TMZ i elf con me he MGMT molec le i one of he ea on h he S o ocol of con in o admini a ion of lo -do e emo olomide a he adj an he a i igni can l e ec i e [22]. TMZ ho ld be e in GB i h a lea 1200 co ie / g RNA of MGMTmRNA in o de o o e come e i ance o TMZ b MGMT.

Be aci mab and h man in e fe on- (hIFN-) a e a ailable fo glioma he a in Ja an. Be aci mab a a o ed fo e in Ja an in 2013 fo bo h ini iall diagno ed and ec en malignan glioma . Be aci mab i e o ed o be mo e e ec i e fo ea ing MGMT- me h la ed mo han nme h la ed mo [23]. Al ho gh ha e III die ha e e o ed ha a co e of be aci mab i h TMZ and adia ion confe ed olonged PFS fo a ien i h GB, OS a no olonged. If e con ide c o -o e ca e in hich Be aci mab a ed a e ec ence in lacebo g o , Be aci mab eem o be e ec i e in elec ed glioma ca e . H man in e fe on (hIFN)- ha been ed fo glioma he a ince 1985, e eciall in Ja an. Al ho gh he e i no e idence on he e ec i ene hIFN- addi ion o TMZ fo olonging PFS and OS, hIFN- a fo nd o do n eg la e MGMT e e ion and a ea o be ef l fo ea ing GB i h nme h la ed MGMT, i.e., in he e ence of high le el of MGMT mRNA [24,25]. A ecen e o ho ed ha in e fe on- / enhance TMZ ac i i again MGMT- o i i e glioma em-like cell [26].

A ne IAT ba ed on he e l of MGMTmRNA an i a ion b eal- ime RT-PCR i ecommended in ini iall diagno ed GB. GB i h le han 1200 co ie / g RNA eem o ha e a good ogno i and no ea men o he han TMZ and adia ion i ed, a lea in he ini ial adj an he a . Fo GB i h a lea 1200 co ie / g RNA, he co- e of Be aci mab and TMZ accom anied b adia ion ma be an e ec i e modali . Al ho gh e en ed o d ha been e o ec i e and on

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References

gene silencing and bene ft from temozolomide in glioblastoma. N Engl J Med

signi fcance of O6-methylguanine-DNA methyltransferase protein expression

Citation:

Motomura K, Natsume A, Kishida Y, Higashi H, Kondo Y, et al. (2011) Benefts of interferon-β and temozolomide combination therapy for newly diagnosed

Identifcation of regions correlating MGMT promoter methylation and gene

Shen D, Guo CC, Wang J, Qiu ZK, Sai K, et al. (2015) Interferon-α/β enhances